2006

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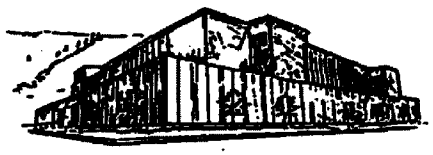

EFFECTS OF EXTENDED EXERCISE AND CARBOHYDRATE FEEDINGS ON SUBSTRATE OXIDATION AND MUSCLE GLYCOGENOLYSIS

By

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B.S. University of Montana, 2002

presented in partial fulfillment of the requirements

for the degree of

Master of Science

The University of Montana

May 2006

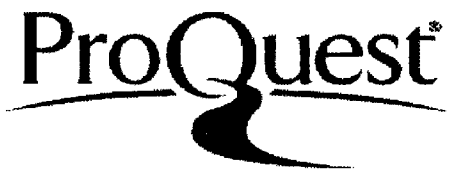
Approved by:

Chairperson

Dean, Graduate School

5-26-06

Date



Wagner, Jamie D., M.S., May 2006 Health and Human Performance

Effects of Extended Exercise and Carbohydrate Feedings on Substrate Oxidation and Muscle Glycogenolysis

Chairperson: Dr. Brent C. Ruby

PURPOSE: The purpose of this study was to examine the effects of exogenous carbohydrate intake during eight hours of prolonged exercise on muscle glycogenolysis and whole-body substrate oxidation. **METHODS:** Eight recreationally-trained males participated in two 8-hour exercise trials. During each hour, subjects repeated 25 minutes cycle ergometer exercise, 5 minutes rest, 25 minutes treadmill exercise, and 5 minutes rest. Each 25 minute exercise segment consisted of two steady state and three interval work bouts. Muscle biopsies were obtained pre- and post-exercise. Blood samples were collected pre-exercise, after hour 4, and post-exercise. Metabolic gases were collected during hours 1, 4, 5, and 8. Every 15 minutes, subjects ingested 150 mL of either a 10% carbohydrate solution (CHO) or a sweetened placebo (PLA). Subjects were provided with a standardized breakfast and lunch. Data were analyzed using repeated-measures ANOVA and statistical significance was set at $p<0.05$. **RESULTS:** There was a significant difference in the rate of muscle glycogenolysis between trials (9.4 ± 2.1 and 13.7 ± 4.6 mmol·kg wet wt.$^{-1}$·hr^{-1} for CHO and PLA, respectively). Rates of whole-body carbohydrate oxidation demonstrated a general maintenance throughout exercise for the CHO trial but showed a decline throughout the PLA trial. Blood glucose and insulin were higher for CHO after hour 4 and post exercise compared to PLA. **CONCLUSION:** The results from this study suggest that regular exogenous carbohydrate feedings during prolonged, intermittent exercise attenuate muscle glycogenolysis while maintaining plasma glucose and insulin concentrations and rates of whole-body carbohydrate oxidation.

Acknowledgements

First and foremost I would like to thank my committee members—Dr. Brent Ruby, Dr. Steven Gaskill, and Dr. Earle Adams. Brent, thanks for your enthusiasm and creativity when it comes to research. You made me excited about this study, and I thank you for trusting me with such an extensive project. Steve, you are an inspiration and a source of support for so many graduate students. Thank you for taking the extra time to help me. Thank you, Earle, for your willingness to jump on board and be a part of this project. I really appreciated your kind words of encouragement.

I also want to thank all my classmates and fellow graduate students. All of you had a part in this project. Thanks to Drew and Mishel for your time and many hours of support as we sat in the lab and watched movies! Just kidding, your help was invaluable—thank you. Thank you also to Cuddy, Ben, and Trevor for their time and effort committed to the project. Thanks to Berd for providing entertainment. What would this world be without Berd and that unforgettable laugh? I also thank Mike for his many hours of help in the lab and willingness to do anything I asked of him.

A special thank you to Steph. I cannot tell you how much I appreciated your help with all the assays, even though you were extremely busy with your own writing. You are a great teacher and friend.

All nine of the subjects provided a different atmosphere which really helped curb the potential monotony that could have developed in this research. Thanks to all of you for your interest and enthusiasm and your willingness to keep going when it wasn't quite so easy. It was a pleasure working with all of you.

Thank you to Crystal for all that you do. You helped me out in so many ways, and it was always a joy to see your smiling face.

To all my friends and family who have always supported my educational endeavors. It's been a long road, yet you've been by my side the whole time. Mom, Dad, and Jessi—words cannot express what you are to me. I love you dearly and would be nothing without you.

Finally, to my rock, my best-friend, my husband, Ben. You have been nothing but supportive and encouraging these past three years. Even when things have been tough financially and I've felt guilty for not working, you would never let me quit and always reminded of the light at the end of the tunnel. Thank you, thank you, thank you for your love, your stability, and your encouragement. You have allowed me to be selfish and follow my dreams—all while sacrificing your own goals and ambitions. Thank you. I love you. And to the new joy in my life, my baby girl—Katelynn. You put things in persepective and make me realize what's truly important. I love you more than I ever thought possible and cannot imagine my life without you.

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Chapter One:

Introduction

Introduction:

The fatigue-delaying and performance-enhancing effects of carbohydrate (CHO) supplementation during physical work and exercise have consistently been demonstrated (26, 78, 98). While the improved work-capacity and performance benefits of ingesting CHO during extended exercise appear conclusive, the mechanism underlying this phenomenon remains unclear. Some researchers argue the delay in the onset of fatigue is due to preservation of muscle glycogen (8, 31, 97, 111), others contend that it is due to maintenance of euglycemia (4, 22, 26, 40), while even others insist that it is not either of these metabolic factors but rather factors associated with central fatigue (17, 73, 91). Most likely, all three of these factors contribute to the complex event of overall fatigue. The prevalence of these mechanisms may depend on other factors such as the type and intensity of exercise, amount, type, and timing of CHO ingestion, pre-exercise nutritional and training status, as well as psychological factors such as motivation and boredom.

Most research has examined moderate to high intensity exercise performed for four or less hours and has established the fatigue-delaying affects of CHO ingestion during activities of such intensities and durations (40, 87, 97, 98, 100). In the fasted state, muscle glycogen may provide as much as 80% of the total CHO oxidized during moderate-high intensity exercise (13). Furthermore, a study by Vollestäd and Blom (104) demonstrates that type I muscle fibers become glycogen depleted after only 60 minutes of *low* intensity exercise. Muscle glycogen is clearly essential as an energy-producing

substrate. Therefore, carbohydrate supplementation becomes even more critical during prolonged activities (i.e. lasting longer than four hours). It cannot be assumed, however, that whole-body and muscle metabolism are similar during exercise lasting 4 hours compared to exercise lasting 6, 8, or even 10 hours. The metabolic response during extended exercise (exercise lasting longer than 4 hours) requires further investigation.

In conjunction with the issue of delaying fatigue via CHO supplementation are the influences of CHO bioavailability and oxidation. Obviously, the maximal performance benefit is going to be elicited by the optimal CHO feeding strategy. Much work has been done in this area to determine the best type, concentration, and amount of CHO to ingest. It seems that a combination of sugar types, rather than a single sugar, promotes the greatest absorption and subsequent oxidation of exogenous carbohydrate sources (61), while increasing concentration does not necessarily correlate with improvement in oxidation (61). These issues also need to be considered during exercise lasting longer than 4 hours.

Thus, while previous research has contributed significantly to our current knowledge of CHO metabolism during exercise, more research is necessary to explain substrate utilization and muscle glycogenolysis during varied-intensity activities lasting longer than four hours.

Statement of the Problem:

Nearly all the research regarding carbohydrate supplementation and substrate oxidation during exercise has been done while examining exercise bouts of four hours or less. Substrate metabolism and muscle glycogen utilization are not as well understood

during exercise lasting longer than four hours. Although there are few physically demanding jobs in today's technologically driven world, those that do exist consist of work shifts much longer than four hours. Issues of fatigue and cognitive ability become especially important late in the work shift. An understanding of metabolism during longer duration activity is necessary and is critical for determining what actions can be taken to attenuate the development of fatigue and keep people cognizant and safe throughout the entire work shift.

Purpose:

The purpose of this research is to determine the effects of carbohydrate supplementation on whole body substrate oxidation, muscle glycogenolysis, and changes in blood glucose, lactate, and insulin concentrations during eight hours of moderate exercise interspersed with higher intensity intervals.

Research Questions:

(1). How does regular carbohydrate supplementation during prolonged, varied intensity exercise affect muscle glycogenolysis?

(2). How does regular carbohydrate supplementation during prolonged, varied intensity exercise affect carbohydrate and fat oxidation (as measured indirectly through metabolic gas collection)?

(3). How does regular carbohydrate supplementation during prolonged, varied intensity exercise affect blood glucose, lactate, and insulin concentrations?

(4). How does regular carbohydrate supplementation during prolonged, varied intensity exercise affect heart rate, rate of perceived exertion (RPE), and cycle cadence?

Research Hypotheses:

(1). Muscle glycogen oxidation will be lower during the CHO trial compared to the placebo trial.

(2). Whole body carbohydrate oxidation will be higher during the CHO trial compared to the placebo trial.

(3). Whole body fat oxidation will be lower during the CHO trial compared to the placebo trial.

(4). Blood glucose concentration will be higher after hours 4 and 8 of exercise during the CHO trial compared to the placebo trial. There will be no difference between the trials in pre-exercise blood glucose concentrations.

(5). Insulin concentration will be higher after hours 4 and 8 of exercise during the CHO trial compared to the placebo trial. There will be no difference between the trials in pre-exercise insulin concentrations.

(6). Lactate concentration will be significantly lower after hours 4 and 8 of exercise during the CHO trial compared to the placebo trial. There will be no difference between the trials in pre-exercise lactate concentrations.

(7). Average heart rate will be lower throughout the CHO trial compared to the placebo trial.

(8). Average RPE will be lower throughout the CHO trial compared to the placebo trial.

(9). Average cycle cadence will be higher during the CHO trial compared to the placebo trial.

Limitations:

There are many factors which influence whole-body metabolism. We are not able to control and/or account for all of them.

Gas exchange is an indirect means of determining ventilatory threshold (VT) and substrate oxidation and can be interpreted only as an estimate. The use of indirect methods, though non-invasive and easily performed, increases measurement error. Muscle biopsy locations are slightly different pre- and post-exercise and may not accurately reflect changes in muscle glycogen concentration.

The use of any instrumentation invites inherent error. In an attempt to limit the occurrence of errors, all researchers will be adequately trained and equipment will be carefully calibrated.

Delimitations:

The results of this study can only be extrapolated to other males of similar age and fitness level.

The exercise was performed on a cycle ergometer and treadmill in a laboratory-controlled environment and may not be transferable to real-life or field-type situations.

Exercise intensity was controlled throughout the trials. In a real-life setting individuals will adapt an exercise intensity to match fatigue and motivation levels.

Definition of Terms:

Carbohydrate (CHO). The treatment subjects received during one of their two trials. Carbohydrate was provided in liquid form at predetermined time points throughout the exercise session.

Placebo (PLA). The treatment subjects received during one of their two trials. An artificially sweetened drink was provided at predetermined time points throughout the exercise session.

Ventilatory Threshold (Tvent). The intensity relative to which the exercise trials were set. VT is an indicator of one's fitness level and is based on nonlinear increases in metabolic gases. VT was estimated using a combination of the v-slope, excess CO_2, and the ventilatory equivalents methods. VT was considered to be the first sustained rise in Ve/VO_2 without a concomitant rise in Ve/VCO_2 (ventilatory equivalents method), concurrent with the first rise in excess CO_2 (excess CO_2 method), and the first break above a slope of 1.0 when VO_2 is plotted versus VCO_2 (v-slope method).

VO_2max. The maximal amount of oxygen an individual consumes during a graded exercise test to volitional exhaustion.

Glycolysis. A cellular energy-producing pathway via the oxidation of glucose.

Glucose. A sugar and the primary source of energy for living organisms. It is the only form of carbohydrate which can undergo glycolysis and ultimate result in energy production.

Lactate. A biproduct of glycolysis which may enter muscle cells and in turn be oxidized to produce energy.

Insulin. A hormone secreted by the pancreas and responsible for regulating glucose levels in the blood. It aids in the uptake of glucose by muscle and other tissues.

Glycogen. The storage form of glucose molecules. Multiple glucose molecules are linked together and stored in either the liver or muscle tissue and degraded for energy as demanded.

Chapter Two:

Review of Literature

Causes of fatigue

Krogh and Lindhardt, in the 1920's, were likely among the first to recognize the importance of CHO as an energy-producing substrate during exercise. They reported that subjects perceived exercise to be easier if it followed a CHO-rich diet versus a high-fat diet (66). Furthermore, Levine and colleagues (70) suggested that low blood glucose levels were likely a cause of fatigue of participants in the 1923 Boston Marathon and commented on the correlation between the "blood sugar level and the physical condition of the runner at the finish". Research by Christensen and Hansen (19) demonstrated a relationship between exercise intensity and the muscle's choice of substrate by assessing indirect measures of whole body gas exchange. While measurements of blood glucose concentrations and estimations of substrate utilization were significant in recognizing the importance of CHO during physical activity, it was studies in the 1960's, which reintroduced the muscle biopsy technique, that indicated the critical role of muscle glycogen as a CHO energy-producing substrate (6, 7).

Subsequent research has produced a debate as to *how* carbohydrate elicits a fatigue-delaying effects. Some researchers contend carbohydrate supplementation during exercise delays the onset of fatigue by slowing muscle glycogen oxidation (8, 31, 97, 111), while others insist it is the maintenance of euglycemia via carbohydrate supplementation that allows a person to continue exercising (4, 22, 40, 79). Yet others

contend fatigue is not purely a metabolic phenomenon, but is due to the breakdown of some central process (17, 27, 28, 29, 73, 91, 105).

Fatigue associated with muscle glycogen concentrations:

The results of a study by Yaspelkis and colleagues (111) led to the conclusion that CHO supplementation could enhance prolonged, variable-intensity exercise via conservation of muscle glycogen. They evaluated muscle glycogen utilization and endurance performance in 7 well-trained cyclists. Subjects performed 3 exercise trials to fatigue with intensity varying between 45% VO_2max and 75% VO_2max. During each of the 3 trials subjects received either a artificially sweetened placebo drink, a 10% CHO liquid solution, or a solid CHO supplement providing 50 g CHO/h. Muscle biopsies were performed before exercise, after the first set of intervals (124 minutes of exercise), and after the second set of intervals (190 minutes of exercise). After the 190-minute biopsy, subjects cycled to exhaustion at 80% VO_2max. The time to fatigue for the liquid and solid CHO trials did not differ, but both were significantly longer than the placebo trial. After 190 minutes muscle glycogen concentrations were significantly greater in the CHO trials than the placebo trial. These researchers ultimately concluded that the increased time to fatigue during the trials in which CHO was provided was due to a reduced dependency on muscle glycogen during the previous 190 minute trial.

Studies which have compared the ergogenic effects of glucose and fructose have also demonstrated the muscle glycogen sparing affect of glucose (8, 31). Total time to exhaustion was significantly longer during continuous exercise at 68% VO_2max when glucose was ingested versus fructose or water (8). Fructose ingestion maintained

euglycemia but failed to attenuate muscle glycogen degradation or improve performance, providing evidence that fatigue is associated with muscle glycogen content, not blood glucose concentration.

Tsintzas has also demonstrated that delayed time to fatigue is associated with conservation of muscle glycogen, however, he contends the sparing of muscle glycogen is limited to type I (slow-twitch) fibers only (97, 98). During one study Tsintzas (98) had 8 recreational male runners complete 2 trials (1-placebo, 1-5.5% CHO solution) at 70% VO_2max until exhaustion. Muscle biopsies performed before exercise, at the time of exhaustion (placebo & CHO), and during the CHO trial, at the time corresponding to fatigue during the placebo trial, illustrated a 25% reduction in muscle glycogen utilization in type I fibers. Furthermore, in both trials, type I but not type II fibers were glycogen depleted at the point of exhaustion—suggesting selective depletion of type I fibers is slower with CHO supplementation. During the CHO trial, blood glucose contributed more to total CHO oxidation, however, no signs or symptoms of hypoglycemia were observed during placebo trial—even at the point of fatigue. Time to exhaustion when CHO was supplemented was ~ 28 minutes longer than when placebo was ingested. Thus, the results of this study suggest fatigue is associated with depletion of glycogen in type I muscle fibers. Similar results were produced in another study by Tsintzas (97) in which cyclists rode for a predetermined time (60 minutes) at 70% VO_2max—again, muscle glycogen (type I fibers only) was conserved when CHO was ingested.

The absolute muscle glycogen concentration may determine the point of fatigue, while the rate of its oxidation may play a role in the regulation of pace during extended exercise. In a study in which cyclists rode for 120 minutes at ~ 73% VO_2max (which

included sprints of 100% peak power output every 20 minutes, followed by a one-hour time trial), time trial performance was improved after carbohydrate loading. End muscle glycogen concentrations, however, were not different between the non-loaded and carbohydrate loaded trials—in fact, they were remarkably similar (18 ± 3 v. 20 ± 3 mmol/kg w/w). The authors concluded that diet-induced changes of muscle glycogen content may have influenced integrated feedback from the periphery and ultimately affected power output and pacing strategies (88).

Fatigue associated with blood glucose concentrations:

A study by Coyle, Coggan, and their colleagues (26) was specifically organized to determine if postponement of fatigue was associated with muscle glycogen sparing. Seven, well-trained, male cyclists performed two trials at ~ 71% VO_2max until exhaustion; the first trial a sweetened placebo drink was administered, and the second trial a glucose polymer drink was administered. The trials were not randomized because the aim of the study was to compare "fed exercise" muscle glycogen oxidation rates and concentrations to muscle glycogen oxidation rates and concentrations after a fatiguing bout of exercise *without* CHO supplementation. Thus, muscle biopsies were performed prior to exercise, after 2 hours of exercise, at the time of fatigue during the placebo trial (for *both* trials), and at the time of fatigue during the fed trial. The average time to fatigue during the placebo trial was about 3 hours; the average time to fatigue during the CHO-fed trial was about 4 hours. Muscle glycogen utilization did not differ between the two trials. The additional exercise when the subjects were fed CHO was performed with

relatively little reliance on muscle glycogen and was attributed to a maintenance of blood glucose concentrations.

Another study by Coggan and Coyle (21) showed delayed fatigue times and restoration of euglycemia by a single CHO feeding late in exercise. They again assert that the increase in CHO oxidation resulting from the restoration/maintenance of euglycemia delayed fatigue.

A study by Arkinstall (4) compared muscle glycogen utilization during different modes of exercise: cycling and running. It was hypothesized that, when fed CHO, muscle glycogen oxidation would be attenuated during running but not during cycling. Subjects cycled and ran at their approximate lactate threshold (LT) for 60 minutes, twice for each mode (4 trials total per subject). During the fed trials subjects ingested a 6.4% solution (8 ml/kg) 10 minutes before exercise and 2 ml/kg at 20 and 40 minutes into exercise. A sweetened placebo was ingested at these same time points during the placebo trials. A tracer (^{14}C) was infused during each trial in order to measure plasma glucose oxidation. In contrast to the researchers' hypothesis, muscle glycogen utilization was not decreased with CHO ingestion during either cycling or running. However, the contribution of plasma glucose to total CHO oxidation was significantly increased during both modes of exercise.

Studies by Hargreaves (40) and Mitchell (79) provide further support for the notion that fatigue is delayed due to maintenance of euglycemia, not attenuation of muscle glycogen oxidation. Hargreaves (40) did not find differences in muscle glycogen concentrations before and following 2 separate, 120-minute cycling trials at 70% VO_2max (one with CHO supplementation, one with a sweetened water drink). A similar

study by Mitchell (79) examined the affects of various concentrations of CHO solutions and found no difference in muscle glycogen utilization. Blood glucose concentrations, however, were significantly increased when the subjects ingested 12 and 18% solutions of CHO.

Other causes of fatigue:

Carbohydrate ingestion may *delay* fatigue, either by maintaining blood glucose concentrations or attenuating muscle glycogen oxidation, but it does not *prevent* fatigue—suggesting factors other than CHO availability eventually cause fatigue. Alternative causes of fatigue have been suggested: depletion of muscle potassium (94), decrease in the force-generating capacity of the myofibril (106), depletion of metabolic intermediates (95), or fatigue of neural/central origin versus metabolic/peripheral (17, 27, 28, 29, 105).

It has been demonstrated that at muscular exhaustion the intracellular potassium concentration [K] is decreased by 20-40 mM. Thus, it is suggested that when the K-gradient across the membrane of the sarcoplasmic reticulum decreases to a certain (threshold) level, excitability of the muscle is impaired, thereby decreasing the contractility of the muscle fibers—indicative of fatigue (94).

Vollestäd (105, 106) has performed experiments which suggest fatigue is due to a decrease in the force-generating capacity of muscle fibers which results from excitation-contraction coupling failure. Subjects performing single-legged quadriceps contractions at 30% maximal voluntary contraction (MVC) for 6 s, with 4 s rest between contractions, showed a 40% decline in MVC after only 30 minutes of exercise. Blood samples,

however, indicated only minor changes in the energy substrates necessary to produce muscle contraction--glycogen, lactate, creatine phosphate (CrP), and adenosine triphosphate (ATP). The decline in MVC was attributed to a failure of excitation-contraction coupling.

Others have suggested that fatigue ensues because of the depletion of certain metabolic intermediates and the corresponding accumulation of other metabolic markers—thus affecting the overall muscle energy balance. The results of studies by Spencer (95) and McConell (78) suggest that carbohydrate supplementation prolongs time to fatigue by attenuating inosine monophosphate (IMP) accumulation (which is associated with muscle glycogen depletion). Spencer had subjects cycle at ~70% VO_2max until exhaustion, while ingesting a placebo solution, and on a second occasion at the same work load and duration, but receiving a 7.5% CHO solution. Subjects were able to cycle for an average of 21.6 minutes longer when receiving the CHO beverage. Muscle biopsies taken before and after exercise revealed the sum of hexose monophosphates (HMP) (intermediates for the Kreb's/TCA Cycle) was higher after exercise with CHO, whereas the accumulation of IMP was markedly reduced. Thus, there is an inverse relationship between HMP and IMP. Fatigue is associated with depletion of HMP and accumulation of IMP, and attenuation of these occurrences and improvement of muscle energy balance, prolongs the onset of fatigue.

The central fatigue hypothesis, though relatively unexplored, suggests that fatigue is associated with an increase in brain serotonin (5-HT) levels. Evidence from studies by Davis (27, 28, 29) suggest a relationship between 5-HT levels and exercise performance. Exercise-induced changes in the ratio between plasma free tryptophan (F-TRP) and

branched-chain amino acids (BCAA) significantly alters brain 5-HT which, in turn, has predictive effects on exercise performance. Essentially, an increase in the f-TRP/BCAA ratio results in an increase in 5-HT, which is associated with central fatigue. A study (27) in which cyclists exercised at their lactate threshold (~ 68% VO_2max) until fatigue while ingesting either a placebo, 6% or 12% CHO solution illustrated a five- to sevenfold increase in f-TRP/BCAA when subjects ingested the placebo solution. The increase in this ratio was significantly attenuated with the CHO solution. Exercise time to fatigue averaged 190, 235, and 234 minutes when receiving the placebo, 6% CHO solution, and 12% CHO solution, respectively.

An interesting study by Carter et al. (17) lends support to the idea that performance improvement via CHO supplementation is related to central mechanisms, not metabolic factors. Trained cyclists received a CHO solution or an identically-tasting placebo. Subjects were not allowed to swallow either drink; it was swished around in the mouth and spat out after a 5 second rinse. Time trial performance, measured in average watt output and time to completion, was significantly improved when receiving the CHO mouth rinse compared to the placebo mouth rinse. These results suggest that there are receptors within the oral cavity which are communicating with the brain and central factors are mediating exercise performance. In a similar study by Carter (18), when the oral cavity was by-passed and glucose was administered via venous infusion, 60-minute time trial performance did not differ from when saline was infused.

Effect of CHO ingestion on muscle glycogenolysis

Muscle glycogen and blood glucose, either endogenously provided by the liver or exogenously derived, comprise the total carbohydrate (CHO) source for ATP production during exercise. Muscle glycogen becomes especially important with increasing exercise intensity. In the fasted state, muscle glycogen may provide as much as 80% of the total CHO oxidized during moderate-high intensity exercise. Thus, despite an increase in glucose oxidation as exercise intensity increases, glycogen is more important as a substrate. With increasing intensity glycogen breakdown is greatly accelerated and glycogen, not glucose, is the major precursor for glycolysis (13). A study by Vollestäd and Blom (104) demonstrates that muscle fibers become glycogen depleted during *low* intensity exercise as well. After only 60 minutes of exercise at 43% VO_2max nearly 50% of type I fibers are glycogen depleted. Another study conducted by Febbraio and Dancey (32) indicated that glycogen availability is a limiting factor during prolonged exercise (155-217 minutes) below the lactate threshold. These observations have led many scientists to agree that intramuscular glycogen stores are the most important limiting factor of exhaustive exercise and as exercise continues, regardless of the intensity level, muscle glycogen stores become depleted and an exogenous source of CHO is necessary to maintain CHO oxidation. Much research has focused on the possibility of conserving muscle glycogen or attenuating its oxidation, via exogenous CHO supplementation, in order to delay the onset of fatigue. The research has produced conflicting results.

Jeukendrup et al. (59) reported muscle glycogen oxidation rates, indirectly estimated from stable isotope tracer infusion (total CHO oxidation minus plasma glucose oxidation), were not attenuated even with glucose ingestion rates as high as 180 grams/hour. Similarly, Flynn et al. (35) measured muscle glycogen concentration

directly with biopsy technique and reported oxidation was not influenced by ingestion of various CHO-% solutions throughout 2 hours of cycling. In contrast, Yaspelkis et al. (111) reported increased time to fatigue and decreased muscle glycogen utilization during continuous, varying-intensity exercise while drinking a 10% liquid CHO solution or eating a solid CHO supplement. These results were repeated in a study where cyclists rode at a constant intensity (~ 68% VO_2max) until exhaustion (8) and in a study where cyclists rode for 90 minutes at 65-70% VO_2max (31).

Inconsistencies and varying results are present even between the results of separate studies conducted by the same scientists. Hargreaves and Briggs (40) had five competitive male cyclists ride for 120 minutes at ~ 70% VO_2max on two different occasions. During the experimental trial subjects ingested 30 g of CHO at 0, 30, 60, and 90 minutes. They ingested a sweetened placebo at these same time points during the other trial. Muscle samples taken pre- and post-exercise revealed no difference in muscle glycogen concentration or oxidation rate. However, another study conducted by Hargreaves, Costill, and Coggan (39) in which subjects were studied during 4 hours of cycling to determine the effect of solid CHO feedings on muscle glycogenolysis and exercise performance revealed significantly lower muscle glycogen utilization during the CHO trial compared to the placebo trial. Contrary to the results of this study, other research conducted by Costill and Coggan has not demonstrated a decrease in muscle glycogenolysis and has attributed any improvement in performance to maintenance of euglycemia (26, 35, 79).

Tsintzas has consistently demonstrated a decrease in muscle glycogenolysis when subjects are fed CHO during exercise (97, 98). His method of glycogen analysis is

sensitive enough to detect changes within fiber-type populations (i.e. slow- or fast-twitch fibers). Consequently, the results of these studies have revealed that conservation of muscle glycogen can occur during low to moderate intensity exercise, but it is generally limited to type I (slow-twitch) fibers. Thus, while mixed glycogen utilization was reduced 25% in a study where males ran at ~ 70% VO_2max until exhaustion, closer analysis revealed this was attributed to a reduction of glycogenolysis within type I fibers and there was no significant difference between trials of glycogenolysis within type II fibers, which showed little glycogenolysis (98).

Research by Kuipers and colleagues suggests that CHO ingestion during exercise that is either intermittent or of low enough intensity may actually stimulate glycogen synthesis in the non-active muscle fibers (64, 68, 69). In one of these studies, seven male cyclists performed intermittent exercise until exhaustion. Upon exhaustion, a muscle biopsy was performed, after which subjects either continued to exercise at 40% VO_2max for 3 hours or rested for 3 hours. During both occasions, subjects ingested 2 liters of a 25% maltodextrin solution. After the 3 hours of exercise or rest another muscle biopsy was taken. Analysis revealed that muscle glycogen concentration increased during both trials from the concentration post exhaustive exercise. However, the glycogen resynthesis was restricted to type II fibers during exercise whereas glycogen resynthesis occurred in both type I and type II fibers during rest (69). Nonetheless, this demonstrates that CHO supplementation may not only provide substrate for ATP production during exercise, but may also contribute to replenishment of glycogen in non-active muscle fibers.

These variable results, both between and within different laboratories, could very well be attributed to differences in testing protocols (type and amount of CHO provided, fed state before exercise, duration and intensity of exercise) and sensitivity of analysis techniques—as pointed out by Tsintzas. By far, the most frequently mode of exercise employed in these studies is cycling. Running and other types of activity elicit a different type of physiological and metabolic response (30, 47, 65), and thus, muscle glycogen utilization differs. Furthermore, metabolic responses are quite different throughout the different ranges of exercise intensity and differ if the exercise is continuous versus intermittent.

Effect of CHO ingestion on hepatic glycogenolysis and gluconeogenesis

Hepatic glycogenolysis is the primary source of endogenous glucose (kidneys contribute negligible amounts). The idea that CHO feedings delay fatigue due to a maintenance of euglycemia suggests an overall reduction in glucose secretion from the liver—exogenous sources are able to either supplement or completely suppress endogenous glucose production (10, 60). Consequently, liver glycogen concentration remains high near the end of exercise, and plasma-glucose concentrations and high rates of total CHO oxidation can be maintained.

In a study by Jeukendrup et al. (60), in addition to concluding that CHO feedings did not attenuate muscle glycogen oxidation, they also concluded that high rates of CHO ingestion can completely suppress endogenous glucose production (EGP). A primed continuous intravenous [6,6-$2H_2$] glucose infusion, along with ^{13}C-enriched ingested glucose allowed the investigators to determine the rates of appearance (Ra) and

disappearance (Rd) from the gut. The rate of EGP could then be calculated as the difference between total Ra and gut Ra. Total endogenous CHO oxidation is a function of liver and muscle glycogenolysis. Taken together, the increase in total CHO oxidation in the CHO trial compared to the placebo trial with the absence of any difference in muscle glycogenolysis between the two trials, researchers were able to conclude that liver glycogenolysis was completely suppressed by CHO feedings during exercise.

Furthermore, studies conducted by Costill et al. (23) and Van Handel et al. (101) measured only slight increases in the glucose mass following CHO ingestion during moderate-intensity exercise—suggesting hepatic glycogenolysis or gluconeogenesis, or both, must be reduced. In fact, the authors concluded that the ingested glucose contributed up to two-thirds of the circulating glucose pool. Similarly, it has been demonstrated that glucose *infusion* during moderate-intensity exercise can at least partially suppress endogenous glucose production in both rats (5, 110) and humans (34, 51).

The effects of ingesting 500 ml/h of a 10% CHO drink on splanchnic (endogenous + exogenous) glucose Ra and plasma glucose oxidation were examined in 17, non-carbohydrate loaded, male cyclists (11). Subjects cycled for 3 hours at ~ 70% VO_2max. The average Ra and oxidation of endogenous glucose was significantly lower when subjects received the CHO versus the placebo. The authors concluded that ingestion of the CHO solution resulted in either a marked liver-glycogen sparing effect or caused a reduction in gluconeogenesis. The exogenous source was sufficient enough to maintain plasma glucose concentrations.

Oxidation of exogenous CHO

Oxidation of exogenous CHO is affected by multiple factors: exercise intensity, duration, training status, muscle glycogen concentration, fed state, and perhaps even mode of exercise. Direct measures and calculations of endogenous versus exogenous glucose oxidation remain difficult due to the variation in measurement techniques. Studies using ^{14}C-glucose have likely underestimated the oxidation of exogenous glucose, while studies using ^{13}C-glucose have probably overestimated it (22, 61). Regardless, multiple studies have been conducted in an attempt to quantify the oxidation of exogenous CHO and explain its interaction with other available substrates.

Exercise duration and exogenous CHO oxidation:

Ahlborg and Felig (2) had subjects cycle for 3 to 3.5 hours after a 12 hour fast. Analysis of glucose changes demonstrated a 16-fold increase in leg-glucose uptake between minutes 40 and 90 of exercise at ~ 60% VO_2max. The increase in blood glucose oxidation with time is likely due, in part, to the decrease in muscle glycogenolysis as muscle glycogen stores become depleted. Thus late in exercise in the fasted state, when muscle glycogen stores are essentially depleted, blood glucose accounts for almost all CHO energy production (21, 26). Considering the demand the central nervous system (CNS) places on the endogenous glucose supply, if an exogenous source of CHO is not provided, muscle activity cannot be continued. Likewise, as exercise proceeds and CHO is ingested, exogenous CHO will contribute proportionately more to total glucose oxidation. A study by Couture et al. (24) examining exogenous and endogenous CHO oxidation during 2 hours of treadmill running (~ 69% VO_2max) exhibited a significant

increase between 80 and 120 minutes in exogenous CHO oxidation (1.25 vs. 2.21 g/min, respectively). Total endogenous glucose oxidation was significantly decreased (37%) when exogenous glucose was oxidized.

Exercise intensity and exogenous CHO oxidation:

Exogenous CHO oxidation is also affected by the relative exercise intensity. During low intensity exercise (~ 30% VO_2max), CHO ingestion results in both hyperglycemia and hyperinsulemia—resulting in a rate of muscle glucose uptake nearly twice that of the same intensity in the fasted state (1). However, CHO intake during moderate intensity exercise (50-75% VO_2max) demonstrates less noticeable alterations in muscle glucose uptake and substrate metabolism. Pirnay et al. (85, 86) reported exogenous CHO oxidation tended to level off between 51% and 64% of VO_2max; with no further increase in exogenous CHO oxidation when the intensity was increased from 60% to 75% VO_2max. They concluded that exogenous glucose is largely oxidized and significantly contributes to the total energy supply; however, at high power outputs, the oxidation rate may level off or even decrease. It is believed that this leveling-off in muscle glucose uptake is primarily due to the suppressed insulin response compared to that at lower intensity levels (20, 21, 109). This is attributed to greater catecholamine stimulation and concurrent sympathetic nervous system (SNS) inhibition at higher exercise intensities (49, 99).

Training status and exogenous CHO oxidation:

It has been noted that training state affects utilization of exogenous CHO. After training, a person is less reliant on CHO metabolism (and more reliant on fat metabolism) at the same absolute workload. The affect of training on CHO metabolism at the same intensity relative to VO_2max or VT is less clear. Most researchers agree, however, that with training a person becomes more tolerable of high glucose levels and is able to more quickly uptake glucose into the muscle due to increased GLUT-4 content and increased capillarization. Training may also result in an improved ability to spare endogenous sources (102).

When Burelle and colleagues (15) examined glucose oxidation at rest and during prolonged exercise, they found that exogenous CHO was oxidized at a higher rate in trained versus sedentary subjects, both at rest and during exercise at the same relative and absolute intensities. This higher exogenous CHO oxidation rate contributed to a better glucose tolerance at rest and a preference for exogenous (vs. endogenous) CHO during exercise in trained subjects.

Another study intending to examine the affect of training status on exogenous CHO oxidation found no difference, after a 6-week training intervention, in ingested glucose oxidation rates at rest, but documented a significant 10% increase in exogenous CHO oxidation rates during 90 minutes of exercise (67). Similarly, a cross-sectional study by Jeukendrup (58) noted a 10% higher rate of exogenous CHO oxidation (though not significant) in trained versus sedentary subjects (50 g vs. 45 g over 120 minutes).

Type of exercise and exogenous CHO oxidation:

Recently it has been suggested that exogenous CHO oxidation may be affected by the type or mode of exercise. It is proposed that CHO ingestion results in more marked elevations in blood glucose and insulin concentrations during running compared with cycling at the same relative exercise intensities. This difference increases muscle glucose uptake and exogenous CHO oxidation rates during running compared to cycling (99).

One study sought to examine the relationship between mode of exercise and exogenous CHO oxidation more closely. It was determined that carbohydrate ingestion during running better maintained plasma glucose concentration than during cycling. However, CHO ingestion increased plasma glucose oxidation relative to total CHO oxidation in both modes (from ~23% to ~35%) (4).

Muscle glycogen content and exogenous CHO oxidation:

It has also been debated as to whether pre-exercise muscle glycogen concentrations affect exogenous CHO oxidation during exercise. Some studies have suggested that lower glycogen levels are associated with a decreased contribution of exogenous CHO oxidation to the total energy expenditure (89).

In contrast to Ravussin's study, which found no difference in the oxidation rates of ingested CHO, despite exogenous CHO contributing relatively less to total energy production, Jeukendrup et al. (56) manipulated pre-exercise glycogen levels and found exogenous glucose oxidation to be 28% lower (36 g vs. 50 g) in those with low glycogen stores compared to those with high glycogen stores. Yet another study found exercising subjects to rely more on exogenous sources after consuming a low CHO diet, when

glycogen availability is presumably low, compared to after consuming a high CHO diet (83).

These conflicting results are likely due to differences in exercise protocols and feeding strategies. It should also be noted that none of these studies performed pre-exercise muscle biopsies and directly measured glycogen content. Muscle glycogen concentration was assumed to be "high" or "low" based on dietary intake.

Limitations / bioavailability of exogenous CHO

In addition to the factors discussed above (exercise duration, intensity, type, training status, and muscle glycogen content), exogenous CHO oxidation can be affected by the type of CHO ingested, the timing of ingestion, and the concentration of the liquid solution. Much research has also focused on the "rate limiting steps" in CHO oxidation, such as physiologic and metabolic factors at the muscle, intestine, or liver.

Type of CHO ingested:

Various types of sugars are available to be ingested: fructose, galactose, maltose, sucrose, maltodextrins, and combinations of these. Differences in the molecular structure affect digestion, absorption, insulin response, and the ultimate ability to be converted into glucose—the only form of sugar which can enter into the muscle and undergo glycolysis and the energy producing pathways.

Fructose is generally more palatable and induces a smaller insulin response than glucose. Studies have documented about a 25% lower oxidation rate of fructose compared to glucose (74, 75). For example, exogenous glucose and fructose oxidation

rates were compared in ten untrained volunteers who exercised for 180 minutes at 45% VO_2max while ingesting either 150 grams of fructose or glucose. Glucose oxidation peaked at about 0.67 g/min, while fructose oxidation only peaked at 0.50 g/min. The lower rate of fructose oxidation is likely due to slower absorption in the intestine and the necessary conversion of fructose into glucose in the liver (61).

Hawley et al. (45) compared the oxidation of maltose and glucose. Trained subjects exercised for 90 minutes at ~ 70% VO_2max while ingesting 180 grams of glucose or maltose. Using radioactive isotopes, exogenous CHO oxidation rates were determined. No significant difference was found in the oxidation rates of glucose compared to maltose (0.9 g/min vs 1.0 g/min, respectively). Furthermore, no differences were found in absorption rates of these two sugars.

Likewise, sucrose has been found to be comparable to glucose in efficacy and oxidation. Moodely and colleagues (80) examined exogenous CHO oxidation rates during 90 minutes of cycling (70% VO_2max) in trained subjects. Subjects ingested solutions of glucose, sucrose, or glucose polymers of various concentrations. Although low (likely a consequence of the methodology), glucose and sucrose oxidation rates both peaked around 0.4 g/min. Higher sucrose oxidation rates, yet similar to those of glucose, were observed by Wagenmakers et al. (107). During 120 minutes of cycling at 65% VO_2max, subjects ingested a total of 145 grams of sucrose in an 8% solution. The peak sucrose oxidation rate was estimated to be about 0.87 g/min—similar to glucose oxidation rates observed in other studies (56, 59, 75, 80).

Maltodextrins, or glucose polymers, are commonly used in sports drinks because of their neutral taste and relatively low osmolarity (61). Oxidation rates of maltodextrin

have been found to be similar to those of glucose (107). In a study where subjects exercised for 80 minutes at 70% VO2max, 220 grams of either a 17% maltodextrin or 17% glucose solution was ingested. Oxidation was found to be similar for the glucose and maltodextrin drinks (42 g vs, 39 g, respectively). The peak oxidation rate for glucose was 0.78 g/min, which was not significantly different from maltodextrin's peak oxidation rate of 0.75 g/min (90).

Researchers have also examined the affect of combining multiple sugar types into a solution. They have hypothesized that a mixture of sugars would reduce competition for transport, and thus, increase total CHO absorption (62). The data suggest that it might, in fact, be helpful in increasing total water and CHO absorption while also maximizing oxidation rates. Jentjens and his colleagues have contributed a significant amount of literature in this area. As discussed below, the majority of research points to a maximum exogenous glucose oxidation rate of 1.0 – 1.1 g/min; however, Jentjens et al. have combined sugars and recorded peak CHO oxidation rates which exceed that of glucose alone (52, 53, 54, 55, 108).

In one study Jentjens et al. (55) sought to compare the oxidation rates of a combined glucose and sucrose solution to the oxidation rates of an isocaloric amount of glucose or sucrose alone. Subjects cycled, on five separate occasions, for 120 minutes at 50% VO_2max, and received either 1.2 g/min of glucose, 1.2 g/min of sucrose (SUC), 0.6 g/min glucose + 0.6 g/min sucrose , 1.2 g/min glucose + 1.2 g/min sucrose , or water. Ultimately, the combination of moderate amounts of glucose and sucrose resulted in 21% higher oxidation rates than the oxidation rate of glucose alone. In a similar study combining glucose and fructose, average exogenous CHO oxidation rates were

significantly higher ($P<0.001$) in 0.6 g/min fructose + 1.2 g/min glucose compared with both moderate (1.2 g/min) and high (1.8 g/min) rates of glucose ingestion alone. This difference represented a nearly 55% higher oxidation rate in fruc+gluc, and the exogenous CHO oxidation rate reached a peak of nearly 1.3 g/min (53). However, in a separate study, no difference was found in peak oxidation rates between glucose ingested alone compared to glucose+maltose (54).

In yet another study, members of Jentjen's lab examined the affect on oxidation rate of combining maltodextrin and fructose (MD+F). Again, peak oxidation rates were ~ 40% higher when MD+F was ingested, compared to ingestion of MD only, and peak oxidation rates of MD+F reached 1.5 g/min (108).

Finally, Jentjens et al. investigated the affect of oxidation rates of combining glucose, sucrose, and fructose (G+S+F). Trained cyclists ingested either water (WAT), 2.4 g/min of glucose (GLU), or 1.2 g/min glucose + 0.6 g/min sucrose + 0.6 g/min fructose (G+S+F), while cycling for 150 minutes at ~ 62% of VO_2max. Peak oxidation rates in the G+S+F trial actually averaged 1.7 g/min—44% higher than peak oxidation rates of GLU (1.18 g/min). Furthermore, researchers were able to determine endogenous CHO oxidation was suppressed more when G+S+F was ingested compared to GLU (52).

Maximal oxidation rate of ingested CHO:

Oxidation efficiency is the ratio of the exogenous CHO oxidation rate to the ingestion rate (61). There is not a direct relationship between the amount of CHO ingested and its oxidation rate (e.g. if a person doubles the amount of CHO ingested in one hour, oxidation rates do not double). Issues of practicality and gastric discomfort

also come into play. Thus, the optimal amount of CHO to ingest is that which will elicit the greatest average oxidation rate, is most practical, and will not result in stomach pain or nausea.

Numerous studies have demonstrated that the maximum exogenous glucose oxidation rate is 1.0 to 1.1 g/min (10, 45, 59, 85), even when large amounts of CHO are ingestion (54, 55, 90, 107).

Factors limiting exogenous CHO oxidation:

There are many possible factors limiting exogenous CHO oxidation: gastric emptying, digestion, intestinal absorption, transport into systemic circulation, rates of hepatic glucose output, and uptake by the muscle. Most evidence suggests that gastric emptying is generally not a major limiting factor during prolonged, moderate-intensity exercise (77, 80, 90). For example, in a study conducted by Massicotte et al. (77) subjects exercised for 120 minutes at 65% VO_2max. At regular intervals, subjects ingested CHO either with or without metoclopramide, a drug known to stimulate gastric emptying. They found no significant difference in the exogenous CHO oxidation rates between the two trials. In other words, metoclopramide did not enhance exogenous CHO oxidation.

In another study, the rate of exogenous CHO oxidation during 90 minutes (70% VO_2max) of cycling exercise was studied. Solutions of 7.5%, 10%, or 15% were ingested at a rate of 100mL every 10 minutes. It was found that gastric emptying actually decreased with increasing concentration; however, delivery to the intestine and exogenous CHO oxidation increased linearly with the increasing CHO concentration.

This indicated that gastric emptying does not limit the rate of exogenous CHO oxidation; some factor subsequent to gastric emptying is regulating oxidation rate (80).

Another possible factor that could restrict exogenous CHO oxidation is the rate at which the glucose is taken up by the muscle. However, like gastric emptying, most researchers have ruled this out as the ultimate limiting factor. Studies by Jeukendrup et al. (59) have estimated that 90 to 95% of the glucose appearing in the bloodstream was taken up and oxidized by the muscle.

The majority of evidence points to intestinal absorption and rate of appearance into the systemic circulation as the primary factors limiting exogenous CHO oxidation. In the Jeukendrup (59) study just mentioned, while 90 to 95% of glucose appearing in bloodstream was oxidized, only about one-third of CHO *ingested* appeared in the bloodstream. A glucose infusion study by Hawley et al. (46) provides strong evidence for intestinal absorption being the key factor dictating exogenous CHO oxidation rate. He had 10 endurance-trained cyclists ride for 125 minutes at 70% VO_2max. Intestinal absorption and hepatic glucose uptake were bypassed by infusing glucose. Bypassing intestinal absorption resulted in glucose oxidation rates considerably above the "normal" oxidation maximum of 1 g/min.

Chapter 3

Methodology

Subjects and Setting:

Subject descriptive data is presented in Table 1. Eight well-trained males served as subjects for the study. Prior to all testing subjects provided written consent via a University Internal Review Board approved informed-consent form. All testing and trials took place in the Human Performance Laboratory on the University of Montana campus in Missoula, MT.

Preliminary Testing:

No more than two weeks prior to the experimental exercise sessions, subjects' descriptive data (percent body fat and maximal aerobic capacity) were collected. Percent body fat was assessed using hydrodensitometry at estimated residual volume. On separate days, maximal aerobic capacity was determined on both the treadmill and cycle ergometer using a graded exercise protocol during which subjects exercised until volitional exhaustion (VO_2max).

Hydrodensitometry: Subjects were fasted for at least three hours prior to the assessment of underwater weight using calibrated electronic scales (Exertech). They were instructed to complete the necessary number of underwater weighing trials until three measurements within 100 grams of each other were obtained. Body density was converted to percent body fat using the Siri equation (93).

Peak Aerobic Capacity (Treadmill): Subjects were fasted for at least three hours prior to initiating the test. After a 10 to 15 minute walking warm-up on the treadmill (Quinton Q65, Seattle, WA), the protocol was initiated at 106.8 $m \cdot min^{-1}$ (4 $mile \cdot hr^{-1}$), 0% grade. The percent grade was increased 1% every 30 seconds until a 20% grade was achieved, after which treadmill speed was increased to 133.8 $m \cdot min^{-1}$ (5 $mile \cdot hr^{-1}$) and progressed 13.38 $m \cdot min^{-1}$ (0.5 $mile \cdot hr^{-1}$) every 30 seconds thereafter until volitional exhaustion (VO_2max) . Subjects received verbal encouragement throughout the test. Expired gases were continuously monitored using a calibrated metabolic system (Parvomedics, Inc., Salt Lake City, UT).

Peak Aerobic Capacity (Cycle Ergometer): Subjects were fasted for no less than three hours prior to the test. After a 10 to 15 minute warm-up at 75 watts on an electronically-braked cycle ergometer (Velotron, Seattle, WA), the protocol was initiated at 0 watts and progressed at a rate of 30 $watts \cdot min^{-1}$ (ramp protocol) until volitional exhaustion (VO_2max). Subjects received verbal encouragement throughout the test. Expired gases were continuously monitored using a calibrated metabolic system (Parvomedics, Inc., Salt Lake City, UT).

Ventilatory Threshold and Intensity Determination: Metabolic gas measurements from each maximal exercise test were analyzed to estimate ventilatory threshold (Tvent) for cycle and treadmill activity. Ventilatory threshold was determined by a combination of three methods: the ventilatory equivalent method, the excess CO_2 method, and the V-slope method according to previously described methodology (37). The exercise

intensity at Tvent for each mode of activity was independently selected by two investigators and then compared. The agreed upon Tvent was used to quantify the percent-grade (treadmill) and power output in watts (cycle ergometer) to establish the exercise intensities for the 8-hr trials (70, 90, 100, 110% of Tvent). The average workloads on the cycle ergometer and treadmill are depicted in Table 2. Each subject's respective workloads were programmed into a computer so that wattage (cycle-ergometer) and percent-grade (treadmill) were automatically adjusted during the exercise trials.

Diet and Activity Specifications: Each subject received dietary instructions for the 24-hour period prior to each 8-hour exercise session. Subjects were prescribed a diet providing 5 g·kg body weight^{-1} carbohydrate and 1.2 g·kg body weight^{-1} protein. The basic diet was developed as a minimum and subjects were asked to record additional food items eaten and to match their diet on the days prior to both 8-hour exercise trials. Subjects were also instructed to maintain their regular exercise/training regimen between the preliminary testing and completion of the second trial. However, they were asked to abstain from any strenuous activity for the 24 hours prior to each 8-hour trial.

Experimental Trials:

The order of the two 8-hour exercise sessions was randomized and completed in a double-blind crossover design, with no less than 7 days between trials. Following a 12-hour fast, subjects reported to the lab at 0615. Upon arrival, subjects were fitted with a chest strap heart-rate monitor and allowed to rest for a period of 10 minutes, after which a

20-mL blood sample was obtained (venopuncture). The blood-handling procedures are described later. A muscle biopsy was obtained from the vastus lateralis under local anesthetic (1% lidocaine). An incision was made through the skin and muscle fascia approximately 12-16 cm proximal to the patella, and a Bergstrom biopsy needle was inserted vertically through the incision into the vastus lateralis. Fresh muscle samples were separated into 25-50 mg sections, placed in cryovials and immediately frozen in liquid nitrogen. At 0715 subjects were provided with a small, standardized breakfast amounting to 3223 kJ (770 kcal), 139 g CHO, 13 g fat, and 23 g protein (PowerBar®, One-Choice nutrition drink, blueberry bagel).

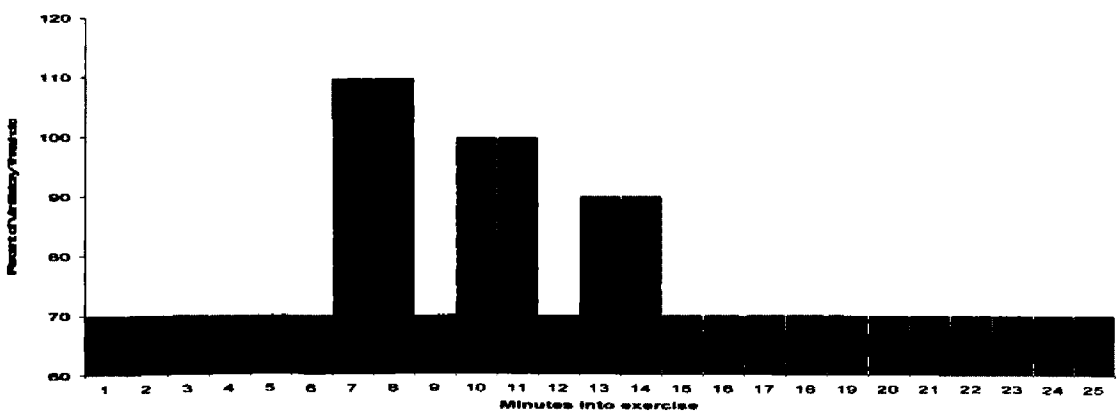
At approximately 0750 subjects were asked to void their bladder and a nude body weight was recorded. Each 8-hour exercise session started at 0800. The exercise protocol is described below. During the *Placebo Trial (PLA)*, subjects consumed 150 ml of an artificially sweetened drink every 15 minutes (minutes 0, 15, 30, 45 of each hour). During the *Carbohydrate Trial (CHO)*, subjects consumed 60 g CHO·hr^{-1} (1003 kJ; 240 kcal) provided in 150 ml of a 10% maltodexdrin solution every 15 minutes, as during the PLA trial.

After the completion of hour 4, subjects were given a 35-minute rest period. During this time a second 20-mL blood sample was obtained. Subjects were then provided with a small standardized lunch providing 3600 kJ (860 kcal), 111 g CHO, 30 g fat, and 36 g protein (Lunchable®, PowerBar®, One-Choice nutrition drink). The final 4 hours of exercise began at 1230.

During hours 1, 4, 5, and 8 metabolic gases were collected during the last 7 minutes on each exercise mode, using the same calibrated metabolic system described

earlier (Parvomedics, Inc., Salt Lake City, UT). Upon completion of the 8th hour of exercise the subject was assisted to a bed, a final 20-mL blood sample was obtained, and the subject was prepped for the post-exercise muscle biopsy. The post-exercise biopsy was obtained from a second incision made approximately 2-2.5 cm proximal to the initial biopsy site.

Exercise protocol: The hour sessions started with 25 minutes of cycling on the same electronically-braked cycle ergometer (Velotron, Seattle, WA) used during the preliminary maximal exercise test. Upon completion of the 25 minutes of cycling, subjects were provided with a 5-minute rest period to allow for changing of shoes and/or use of the lavatory prior to transitioning to the treadmill. The 25 minutes of treadmill walking were performed on the same motorized treadmill (Quinton Q65, Seattle, WA) used during the preliminary maximal exercise test. After completing the 25 minutes of exercise on the treadmill, subjects were again provided with a 5-minute rest period to allow for changing of shoes and/or use of the lavatory prior to transitioning back to the cycle ergometer. Cycling and treadmill walking were repeated for the 8-hour periods. Figure 1 depicts the 25-minute intensity breakdown for each mode of exercise.



The exercise was initiated with six minutes of cycling/walking at 70% Tvent, followed by a sequence of three, two-minute intervals completed at 110, 100, and 90% Tvent, respectively. Each interval was followed by one-minute at 70% Tvent. The remaining 10 minutes of exercise were completed at 70% Tvent.

Procedures

Metabolic Gas Collection: Metabolic gases were collected during 70% Tvent exercise throughout the 8-hour exercise session during the time points previously specified. The steady-state values VO_2 and VCO_2 measurements were used to calculate whole-body carbohydrate and fat oxidation (36).

Heart Rate and RPE: Heart rate (HR) was continuously monitored and minute averages were recorded via a chest-strap transmitter and a receiver (Polar). The data was later downloaded and imported into an Excel spreadsheet for analysis. The average heart rate during the last minute of the 110% Tvent interval and the average heart rate during the last five minutes at 70% Tvent are reported. RPE was recorded during hours 1, 4, 5, and 8 on both modes of exercise at each intensity. During the last minute of each interval (minutes 7, 10, and 13) and minute 22 (70% Tvent), subjects were instructed to look at the 6-20 Borg RPE scale and respond with how hard they felt they were working overall.

Blood Samples: Each of the three blood samples (pre, mid, and post 8-hours) were collected from an anticubital vein into four 5-mL Vacutainer vials (20-mL each collection). The samples remained at room temperature for 15 minutes, after which, they

were centrifuged at 4000 rev·min^{-1}, 4°C for 10 minutes. Serum was separated into designated cryovials and frozen at -80°C until subsequent analysis of glucose, insulin, and lactate concentrations.

Glucose, Insulin, and Lactate Analysis: Blood samples for glucose were analyzed in duplicate using an enzymatic spectrophotometric method (Infinity glucose (HK) liquid stable reagent, ThermoTrace Ltd.). Blood samples for insulin were analyzed in duplicate using an enzymatic spectrophotometric Elisa method (EIA2935, DRG International). Similarly, samples for lactate were analyzed in duplicate using an enzymatic spectrophotometric method (71).

Muscle Glycogen Analysis: Muscle tissue samples for glycogen concentration were analyzed using an enzymatic spectrophotometric method (Infinity glucose (HK) liquid stable reagent, ThermoTrace Ltd.). Upon removal from the -80°C freezer 20-25 μg samples were weighed and homogenized in 1 mL of a 1 N HCL solution using a manual mortar and pestle tissue grinder. Ground samples were incubated for three hours at 95.6°C. After incubation, the pH was normalized by adding 0.5 mL of 1 N NaOH to 0.5 mL of boiled tissue sample. Samples were analyzed in triplicate against known glycogen and glucose controls as previously described (92).

Research Design and Statistical Analyses

Urinary nitrogen and changes in body weight were analyzed using dependent t-tests. All other variables were compared using 2-way repeated-measures ANOVA using

a series of a-priori planned comparisons. Statistical significance was set at $p<0.05$. All values are reported as means ± SD.

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Manuscript

Submitted for publication in the journal of *Medicine and Science in Sports and Exercise*

CARBOHYDRATE FEEDINGS REDUCE MUSCLE GLYCOGENOLYSIS DURING ULTRA-ENDURANCE EXERCISE

Running Title: CHO reduces muscle glycogenolysis

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ABSTRACT

PURPOSE: The purpose of this study was to examine the effects of exogenous carbohydrate intake during eight hours of prolonged exercise on muscle glycogenolysis and whole-body substrate oxidation. **METHODS:** Eight well-trained males participated in two 8-hour exercise trials. During each hour, subjects repeated 25 minutes cycle ergometer exercise, 5 minutes rest, 25 minutes treadmill exercise, and 5 minutes rest. Each 25 minute exercise segment consisted of two steady state and three interval work bouts. Muscle biopsies were obtained pre- and post-exercise. Blood samples were collected pre-exercise, after hour 4, and post-exercise. Metabolic gases were collected during hours 1, 4, 5, and 8. Every 15 minutes, subjects ingested 150 mL of either a 10% carbohydrate solution (CHO) or a sweetened placebo (PLA). Subjects were provided with a standardized breakfast and lunch. Data were analyzed using repeated-measures ANOVA and statistical significance was set at $p<0.05$. **RESULTS:** There was a significant difference in the rate of muscle glycogenolysis between trials (9.4 ± 2.1 and 13.7 ± 4.6 mmol·kg wet wt.$^{-1}$·hr^{-1} for CHO and PLA, respectively). Rates of whole-body carbohydrate oxidation demonstrated a general maintenance throughout exercise for the CHO trial but showed a decline throughout the PLA trial. Blood glucose and insulin were higher for CHO after hour 4 and post exercise compared to PLA. **CONCLUSION:** The results from this study suggest that regular exogenous carbohydrate feedings during prolonged, intermittent exercise attenuate muscle glycogenolysis while maintaining plasma glucose and insulin concentrations and rates of whole-body carbohydrate oxidation.

KEY WORDS: substrate oxidation, muscle glycogenolysis, prolonged exercise

INTRODUCTION

Paragraph 1: The fatigue-delaying and performance-enhancing effects of carbohydrate (CHO) supplementation during physical work and exercise have consistently been demonstrated (4, 18, 26). While the improved work-capacity and performance benefits of ingesting CHO during exercise appear conclusive, the mechanism underlying this phenomenon remains unclear. While some researchers have suggested that fatigue is postponed due to the preservation of muscle glycogen (2, 6, 25, 30), others contend that it is due to maintenance of euglycemia (1, 4, 12), while others suggest a central fatigue mechanism (3, 22). Most likely, all three of these factors contribute to the complex event of overall fatigue during prolonged muscular work. The combination of these proposed mechanisms may depend on other factors such as the mode and intensity of exercise, amount, type, and timing of CHO ingestion, pre-exercise nutritional and training status, as well as psychological factors such as motivation and boredom. Additionally, most research has examined only moderate to high intensity exercise performed for four or less hours (12, 20, 25, 26, 27). It cannot be assumed that whole-body and muscle metabolism are similar during exercise lasting up to 4 hours compared to exercise lasting much longer.

Paragraph 2: In the fasted state, muscle glycogen provides a majority of the total CHO oxidized during moderate-high intensity exercise. However, it has been demonstrated that CHO feedings could reduce muscle glycogen depletion during exercise (11), and that this reduced muscle glycogenolysis is associated with a delayed time to fatigue. Some researchers contend this sparing of muscle glycogen occurs primarily in type I fibers (25,

26, 28, 30), which may become glycogen depleted after only 60 minutes of *low* intensity exercise (28), and the selective depletion of these fibers is slower when exogenous CHO is provided (25, 26). It has also been shown that time trial performance is improved after carbohydrate loading, despite no differences in end muscle glycogen concentrations (21). This suggests that the underlying rate of CHO oxidation may play a role in the regulation of pace during extended exercise, while the absolute glycogen concentration may determine the point of fatigue. Therefore, regardless of the combined causes of fatigue, muscle glycogen is clearly essential as an energy substrate, which suggests regular carbohydrate supplementation may be even more critical during prolonged activities (i.e. lasting longer than four hours). Furthermore, in conjunction with a reduction in muscle glycogenolysis, exogenous CHO may also maintain rates of whole-body CHO oxidation rates (13, 14), which is critical if a work rate is expected to be sustained. Thus, while previous research has contributed significantly to our current knowledge of CHO metabolism during shorter duration, higher intensity exercise, more research is necessary to explain substrate utilization and muscle glycogenolysis during extended sport and work lasting longer than four hours.

Paragraph 3: The purpose of this study was to determine the effects of carbohydrate supplementation on whole-body substrate oxidation and muscle glycogenolysis during eight hours of intermittent, moderate-intensity exercise interspersed with higher intensity intervals. This protocol was designed to simulate real-life extended work patterns. We hypothesized that whole-body carbohydrate oxidation would be better maintained during

the CHO trial compared to the placebo trial and that the oxidation of muscle glycogen would be lower during the CHO trial compared to the placebo trial.

METHODS

Subjects and Setting:

Paragraph 4: Subject descriptive data is presented in Table 1. Eight well-trained males served as subjects for the study. Prior to all testing subjects provided written consent via a University Internal Review Board approved informed-consent form. All testing and trials took place in the Human Performance Laboratory on the University of Montana campus in Missoula, MT.

(Suggest Table 1 here)

Preliminary Testing:

Paragraph 5: No more than two weeks prior to the experimental exercise sessions, subjects' descriptive data (percent body fat and maximal aerobic capacity) were collected. Percent body fat was assessed using hydrodensitometry at estimated residual volume. On separate days, maximal aerobic capacity was determined on both the treadmill and cycle ergometer using a graded exercise protocol during which subjects exercised until volitional exhaustion (VO_2max).

Paragraph 6: Hydrodensitometry: Subjects were fasted for at least three hours prior to the assessment of underwater weight using calibrated electronic scales (Exertech). They were instructed to complete the necessary number of underwater weighing trials until three measurements within 100 grams of each other were obtained. Body density was converted to percent body fat using the Siri equation (24).

Paragraph 7: Peak Aerobic Capacity (Treadmill): Subjects were fasted for at least three hours prior to initiating the test. After a 10 to 15 minute walking warm-up on the treadmill (Quinton Q65, Seattle, WA), the protocol was initiated at 106.8 $m \cdot min^{-1}$ (4 $mile \cdot hr^{-1}$), 0% grade. The percent grade was increased 1% every 30 seconds until a 20% grade was achieved, after which treadmill speed was increased to 133.8 $m \cdot min^{-1}$ (5 $mile \cdot hr^{-1}$) and progressed 13.38 $m \cdot min^{-1}$ (0.5 $mile \cdot hr^{-1}$) every 30 seconds thereafter until volitional exhaustion (VO_2max) . Subjects received verbal encouragement throughout the test. Expired gases were continuously monitored using a calibrated metabolic system (Parvomedics, Inc., Salt Lake City, UT).

Paragraph 8: Peak Aerobic Capacity (Cycle Ergometer): Subjects were fasted for no less than three hours prior to the test. After a 10 to 15 minute warm-up at 75 watts on an electronically-braked cycle ergometer (Velotron, Seattle, WA), the protocol was initiated at 0 watts and progressed at a rate of 30 $watts \cdot min^{-1}$ (ramp protocol) until volitional exhaustion (VO_2max). Subjects received verbal encouragement throughout the test. Expired gases were continuously monitored using a calibrated metabolic system (Parvomedics, Inc., Salt Lake City, UT).

Paragraph 9: Ventilatory Threshold and Intensity Determination: Metabolic gas measurements from each maximal exercise test were analyzed to estimate ventilatory threshold (Tvent) for cycle and treadmill activity. Ventilatory threshold was determined by a combination of three methods: the ventilatory equivalent method, the excess CO_2 method, and the V-slope method according to previously described methodology (10).

The exercise intensity at Tvent for each mode of activity was independently selected by two investigators and then compared. The agreed upon Tvent was used to quantify the percent-grade (treadmill) and power output in watts (cycle ergometer) to establish the exercise intensities for the 8-hr trials (70, 90, 100, 110% of Tvent). The average workloads on the cycle ergometer and treadmill are depicted in Table 2. Each subject's respective workloads were programmed into a computer so that wattage (cycle-ergometer) and percent-grade (treadmill) were automatically adjusted during the exercise trials.

(Suggest Table 2 here)

Paragraph 10: Diet and Activity Specifications: Each subject received dietary instructions for the 24-hour period prior to each 8-hour exercise session. Subjects were prescribed a diet providing 5 g·kg body weight^{-1} carbohydrate and 1.2 g·kg body weight^{-1} protein. The basic diet was developed as a minimum and subjects were asked to record additional food items eaten and to match their diet on the days prior to both 8-hour exercise trials. Subjects were also instructed to maintain their regular exercise/training regimen between the preliminary testing and completion of the second trial. However, they were asked to abstain from any strenuous activity for the 24 hours prior to each 8-hour trial.

Experimental Trials:

Paragraph 11: The order of the two 8-hour exercise sessions was randomized and completed in a double-blind crossover design, with no less than 7 days between trials.

Following a 12-hour fast, subjects reported to the lab at 0615. Upon arrival, subjects were fitted with a chest strap heart-rate monitor and allowed to rest for a period of 10 minutes, after which a 20-mL blood sample was obtained (venopuncture). The blood-handling procedures are described later. A muscle biopsy was obtained from the vastus lateralis under local anesthetic (1% lidocaine). An incision was made through the skin and muscle fascia approximately 12-16 cm proximal to the patella, and a Bergstrom biopsy needle was inserted vertically through the incision into the vastus lateralis. Fresh muscle samples were separated into 25-50 mg sections, placed in cryovials and immediately frozen in liquid nitrogen. At 0715 subjects were provided with a small, standardized breakfast amounting to 3223 kJ (770 kcal), 139 g CHO, 13 g fat, and 23 g protein (PowerBar®, One-Choice nutrition drink, blueberry bagel).

Paragraph 12: At approximately 0750 subjects were asked to void their bladder and a nude body weight was recorded. Each 8-hour exercise session started at 0800. The exercise protocol is described below. During the *Placebo Trial (PLA)*, subjects consumed 150 ml of an artificially sweetened drink every 15 minutes (minutes 0, 15, 30, 45 of each hour). During the *Carbohydrate Trial (CHO)*, subjects consumed 60 g $CHO \cdot hr^{-1}$ (1003 kJ; 240 kcal) provided in 150 ml of a 10% maltodexdrin solution every 15 minutes, as during the PLA trial.

Paragraph 13: After the completion of hour 4, subjects were given a 35-minute rest period. During this time a second 20-mL blood sample was obtained. Subjects were then provided with a small standardized lunch providing 3600 kJ (860 kcal), 111 g CHO, 30 g

fat, and 36 g protein (Lunchable®, PowerBar®, One-Choice nutrition drink). The final 4 hours of exercise began at 1230.

Paragraph 14: During hours 1, 4, 5, and 8 metabolic gases were collected during the last 7 minutes on each exercise mode, using the same calibrated metabolic system described earlier (Parvomedics, Inc., Salt Lake City, UT). Upon completion of the 8th hour of exercise the subject was assisted to a bed, a final 20-mL blood sample was obtained, and the subject was prepped for the post-exercise muscle biopsy. The post-exercise biopsy was obtained from a second incision made approximately 2-2.5 cm proximal to the initial biopsy site.

Exercise protocol:

Paragraph 15: The hour sessions started with 25 minutes of cycling on the same electronically-braked cycle ergometer (Velotron, Seattle, WA) used during the preliminary maximal exercise test. Upon completion of the 25 minutes of cycling, subjects were provided with a 5-minute rest period to allow for changing of shoes and/or use of the lavatory prior to transitioning to the treadmill. The 25 minutes of treadmill walking were performed on the same motorized treadmill (Quinton Q65, Seattle, WA) used during the preliminary maximal exercise test. After completing the 25 minutes of exercise on the treadmill, subjects were again provided with a 5-minute rest period to allow for changing of shoes and/or use of the lavatory prior to transitioning back to the cycle ergometer. Cycling and treadmill walking were repeated for the 8-hour periods. Figure 1 depicts the 25-minute intensity breakdown for each mode of exercise.

(Suggest Figure 1 here)

The exercise was initiated with six minutes of cycling/walking at 70% Tvent, followed by a sequence of three, two-minute intervals completed at 110, 100, and 90% Tvent, respectively. Each interval was followed by one-minute at 70% Tvent. The remaining 10 minutes of exercise were completed at 70% Tvent.

Paragraph 16: Metabolic Gas Collection: Metabolic gases were collected during 70% Tvent exercise throughout the 8-hour exercise session during the time points previously specified. The steady-state values VO_2 and VCO_2 measurements were used to calculate whole-body carbohydrate and fat oxidation (9).

Paragraph 17: Heart Rate and RPE: Heart rate (HR) was continuously monitored and minute averages were recorded via a chest-strap transmitter and a receiver (Polar). The data was later downloaded and imported into an Excel spreadsheet for analysis. The average heart rate during the last minute of the 110% Tvent interval and the average heart rate during the last five minutes at 70% Tvent are reported. RPE was recorded during hours 1, 4, 5, and 8 on both modes of exercise at each intensity. During the last minute of each interval (minutes 7, 10, and 13) and minute 22 (70% Tvent), subjects were instructed to look at the 6-20 Borg RPE scale and respond with how hard they felt they were working overall.

Paragraph 18: Blood Samples: Each of the three blood samples (pre, mid, and post 8-hours) were collected from an anticubital vein into four 5-mL Vacutainer vials (20-mL

each collection). The samples remained at room temperature for 15 minutes, after which, they were centrifuged at 4000 rev·min^{-1}, 4°C for 10 minutes. Serum was separated into designated cryovials and frozen at -80°C until subsequent analysis of glucose, insulin, and lactate concentrations.

Paragraph 19: Glucose, Insulin, and Lactate Analysis: Blood samples for glucose were analyzed in duplicate using an enzymatic spectrophotometric method (Infinity glucose (HK) liquid stable reagent, ThermoTrace Ltd.). Blood samples for insulin were analyzed in duplicate using an enzymatic spectrophotometric Elisa method (EIA2935, DRG International). Similarly, samples for lactate were analyzed in duplicate using an enzymatic spectrophotometric method (17).

Paragraph 20: Muscle Glycogen Analysis: Muscle tissue samples for glycogen concentration were analyzed using an enzymatic spectrophotometric method (Infinity glucose (HK) liquid stable reagent, ThermoTrace Ltd.). Upon removal from the -80°C freezer 20-25 μg samples were weighed and homogenized in 1 mL of a 1 N HCL solution using a manual mortar and pestle tissue grinder. Ground samples were incubated for three hours at 95.6°C. After incubation, the pH was normalized by adding 0.5 mL of 1 N NaOH to 0.5 mL of boiled tissue sample. Samples were analyzed in triplicate against known glycogen and glucose controls as previously described (23).

Paragraph 21: Research Design and Statistical Analyses: Change in body weight was analyzed using dependent t-tests. All other variables were compared using 2-way

repeated-measures ANOVA using a series of a-priori planned comparisons. Statistical significance was set at $p<0.05$. All values are reported as means ± SD.

RESULTS

Paragraph 22: Body weight decreased across both trials, but there was no significant difference between trials (Δ CHO = -1.7 ± 1.0; Δ PLA = - 2.1 ± 0.8; $p<0.05$). Self-selected cycling cadence was not significantly different between the CHO and PLA trials (80.2 ± 9.8 and 80.7 ± 12.4 rev·min^{-1} for CHO and PLA, respectively; $p<0.05$).

Heart Rate

Paragraph 23: The average heart rate during 70% Tvent and 110% Tvent were not significantly different between trials on either the cycle-ergometer or the treadmill ($p<0.05$). However, during both trials heart rate increased across time for each mode and workload. Data are presented in Tables 3a and 3b.

Rates of Perceived Exertion

Paragraph 24: There were no signficant differences between CHO and PLA in RPE at any time point for either the cycle ergometer or treadmill ($p<0.05$). RPE increased across time for each mode and workload. Data are presented in Tables 4a and 4b.

Blood Markers

Paragraph 25: Lactate: There were no significant differences between trials in the pre-, mid-, and post-exercise lactate concentrations ($p<0.05$). The overall average lactate concentration during CHO was lower than the overall average lactate concentration throughout PLA (1.83 ± 0.42; 2.14 ± 0.77 mmol·L^{-1} for CHO and PLA, respectively; $p<0.05$). The average post-exercise lactate concentration of both trials was higher than

the average pre-exercise lactate concentration of both trials (1.70 ± 0.40; 2.30 ± 0.69 mmol·L $^{-1}$ for pre and post, respectively; $p<0.05$). Blood lactate concentrations are presented in Figure 2.

Paragraph 26: Glucose: Blood glucose concentrations were significantly higher during the CHO trial at mid- and post-exercise compared to the placebo trial ($p<0.05$). During the CHO trial, plasma glucose concentrations were significantly higher mid- and post-exercise compared to pre-exercise ($p<0.05$). In contrast, during the placebo trial, the plasma glucose concentrations were significantly lower post-exercise compared to pre-exercise ($p<0.05$). Blood glucose concentrations are presented in Figure 3.

Paragraph 27: Insulin: Insulin concentrations were significantly higher during the CHO trial at mid- and post-exercise compared to PLA ($p<0.05$). During the CHO trial, insulin concentrations were significantly increased mid- and post-exercise compared to pre-exercise ($p<0.05$). In contrast, during the placebo trial, the insulin concentration was significantly lower post-exercise compared to pre-exercise ($p<0.05$). Insulin concentrations are presented in Figure 4.

Substrate oxidation

Paragraph 28: Carbohydrate oxidation: The calculated rates of whole-body carbohydrate oxidation during both cycle and treadmill exercise were significantly higher during CHO compared to PLA for all hours of measurement after hour 1 ($p<0.05$). Within the placebo trial, hours 4, 5, and 8 all exhibited significantly lower rates of CHO

oxidation compared to hour 1 ($p<0.05$). In contrast, there were no differences in CHO oxidation across time during the CHO trial, except during hour 8 of treadmill exercise where there was a small decline ($p<0.05$). (See Figure 5a and 5b).

Paragraph 29: Fat oxidation: The calculated rates of whole-body fat oxidation during both cycle and treadmill exercise were significantly lower during CHO compared to PLA for all hours of measurement after hour 1 ($p<0.05$). During PLA, fat oxidation was significantly elevated above hour 1 at hours 4, 5, and 8 ($p<0.05$). During CHO, fat oxidation was significantly lower at hour 5 compared to hour 1 during cycling, but not during treadmill exercise, and significantly higher at hour 8 compared to hour 1 for both the cycle and treadmill ($p<0.05$). (See Figure 6a and 6b).

Muscle Glycogenolysis

Paragraph 30: Both CHO and PLA demonstrated a significant decrease in muscle glycogen concentration across the 8-hour exercise trial ($p<0.05$). There were no significant differences between CHO and PLA in the pre-exercise muscle glycogen concentrations. However, post-exercise muscle glycogen values were significantly lower for PLA compared to CHO ($p<0.05$) (Figure 7). The calculated rate of muscle glycogenolysis was significantly lower for CHO vs. PLA (9.4 ± 2.1 and 13.7 ± 4.6 mmol·kg wet wt.$^{-1}$·hr^{-1}, respectively; $p<0.05$).

DISCUSSION

Paragraph 31: The effects of CHO supplementation on muscle metabolism and substrate oxidation have not been thoroughly investigated during physical activities lasting longer than four hours. This study presents a unique opportunity to examine whole-body metabolism and muscle glycogenolysis during prolonged, varied-intensity exercise. The premise behind this investigation was to simulate a physical work day under controlled laboratory conditions. Thus, the intensity was set relative to each individual's Tvent, the routine was interspersed with high and low intensity work bouts, different modes of activity were employed, and short rest periods were provided—the primary criteria ultimately characterizing how an individual works in the field. We investigated whether an exogenous CHO source would alter muscle glycogenolysis during the course of eight hours of exercise. Prior research has demonstrated that CHO supplementation improves performance (4, 15, 18, 26) and may decrease muscle glycogen utilization during shorter, steady-paced activities (2, 6, 25, 30). The results of this study provide evidence that these same responses occur during longer, varied-intensity activities with short rest periods.

Paragraph 32: In the present investigation an average of 1 $g \cdot min^{-1}$ of exogenous CHO was provided and was sufficient enough to maintain whole-body CHO oxidation rates throughout much of the day—averaging 1.97 ± 0.39 $g \cdot min^{-1}$ across the day and peaking at 2.07 ± 0.17 $g \cdot min^{-1}$ in hour 5 (following lunch) during cycling exercise. These values are similar to those of Jeukendrup et al. who provided 1.5 $g \cdot min^{-1}$ of exogenous CHO and observed average total CHO oxidation rates of approximately 2 $g \cdot min^{-1}$ over 5 hours of

exercise (15). Jeukendrup et al.'s subjects exercised at an average of 58% VO_2max, as compared to the current study in which subjects averaged 50% VO_2max during the 70% Tvent, steady-state segments. The slightly higher values of CHO oxidation rates found by Jeukendrup et al. are probably due to the higher exercise intensity. Importantly, exogenous CHO supplied at 1 to 1.5 $g \cdot min^{-1}$ was sufficient to maintain whole-body CHO oxidation over the duration of both studies.

Paragraph 33: During the treadmill portion of the exercise trial, rates of whole-body CHO oxidation averaged 1.92 ± 0.31 $g \cdot min^{-1}$ during hours 1, 4, and 5, but dropped to 1.70 ± 0.43 $g \cdot min^{-1}$ during the final hour. Unlike the cycle, total CHO oxidation on the treadmill was not maintained throughout the entire 8 hours of exercise, showing a slight, but significant, drop during the last collection period. This may indicate a mode specific difference in the metabolic responses to exogenous CHO ingestion, as has been previously suggested (1, 5) or it may be a function of time, since treadmill walking followed cycling. However, over the course of eight hours of exercise, it is not likely that an additional 25 minutes of treadmill walking provides the complete explanation for the significant drop in CHO oxidation.

Paragraph 34: The most significant finding from this study is the decreased rate of muscle glycogenolysis when CHO was supplemented at 60 $g \cdot hr^{-1}$ (1 $g \cdot min^{-1}$) for 8 hours compared to a placebo solution. This, in combination with the overall maintenance of whole-body CHO oxidation, indicates a sparing of muscle glycogen with a concomitant increased reliance on exogenous CHO sources as the exercise proceeds. These findings

agree with those from previous research which has documented a reduced dependency on muscle glycogen stores as exercise continues and an exogenous source of CHO is provided (2, 6, 11, 25, 26, 30). Yaspelkis et al. (30) demonstrated that after 190 minutes of variable-intensity exercise, muscle glycogen concentrations were better maintained when subjects were fed CHO versus a placebo. Moreover, subsequent time to exhaustion averaged 25 minutes longer during the CHO trial—indicating a slower rate of muscle glycogenolysis and a reduced dependency on muscle glycogen during the previous 190 minutes of exercise. Despite the duration of our exercise trials being much longer, our data agrees with that of Yaspelkis et al. and demonstrates the potential glycogen sparing effects of exogenous CHO when the exercise is prolonged, the intensity is varied, and short rest periods are provided.

Paragraph 35: During the PLA trial, the rate of muscle glycogenolysis was approximately 45% higher compared to the CHO trial, while the rates of whole-body CHO oxidation (during cycling) were 26, 20 and 63% lower at hours 4, 5, and 8, respectively. Similarly, the rates of whole-body CHO oxidation during the treadmill portion of the exercise were 36, 32, and 89% lower during the PLA trial for hours 4, 5, and 8, respectively. These data clearly demonstrate that *exogenous* CHO was the primary substrate contributing to whole-body CHO oxidation, and while muscle glycogenolysis was greatly attentuated by CHO supplementation, the effects on whole-body CHO oxidation were more predominant.

Paragraph 36: The influence on blood glucose concentrations by exogenous CHO across these long trials was another important result. Not only was blood glucose maintained, but it rose significantly compared to pre-exercise values throughout the CHO trial. These results are in contrast to previous research (4, 12, 19) which was specifically designed to determine if postponement of fatigue was due to muscle glycogen sparing or maintenance of euglycemia. Since our exercise and feeding protocols are different from these studies, direct comparisons cannot be made; however, the subjects in the prior studies did not exhibit a difference in muscle glycogen utilization. The major contributing difference between our study and those mentioned is likely the lower intensity of our exercise (~50% VO2max vs. ~70% VO2max in the other studies). In our study, the significant increase in blood glucose concentrations, in combination with the decreased rate of muscle glycogenolysis, again suggests that perhaps a feeding strategy which provides at least 60 g CHO·hr^{-1} may not only maintain euglycemia, but also maintain total CHO oxidation with less reliance on muscle glycogen stores during extended, varied-intensity exercise.

Paragraph 37: Since the rate of endogenous CHO oxidation (Ra and Rd glucose) was not measured in the current study, whether or not the exogenous source of glucose completely suppressed hepatic glycogenolysis cannot be ascertained. However, considering both trials were performed following a 12-hour fast, and the breakfast and lunch only provided about 6823 kJ (1630 kcal), it is a reasonable assumption that the contribution from hepatic glycogen was similar between trials and was not a major contributor to the overal difference in oxidized CHO. Furthermore, the maintenance of

total CHO oxidation in conjunction with the decreased rate of muscle glycogenolysis during the CHO trial, suggests exogenous CHO *alone* may have maintained whole-body CHO oxidation during the later hours of exercise. Future research should aim to investigate the endogenous glucose response to long-duration, varied-intensity activity. However, the nature of stable isotopic tracer infusion methodology presents difficulties during work bouts of this duration and those difficulties will need to be addressed in order to investigate glucose kinetics during prolonged work.

Paragraph 38: The insulin response in our study paralled the changes in blood glucose—demonstrating a significant rise throughout the 8-hour work period during the CHO trial. Interestingly, the blood glucose and insulin response in this study are in contrast to the findings from other studies in which values tended to peak around 30 minutes into exercise and then steadily decline (8, 12, 26). This difference is most likely due to the lower exercise intensity required for the extended duration of our trials. Lower intensity exercise elicits a more pronounced insulin response than higher intensity exercise (4, 12, 19), thereby increasing glucose uptake, especially during rest periods. Most prior studies have examined exercise performed at higher intensities, which tends to blunt the insulin response. Furthermore, it has been suggested that both a hyperglycemic and hyperinsulimic response are necessary in order to reduce muscle glycogen utilization (30). Our blood glucose and insulin data do agree with those of Yaspelkis et al. (30) in the study described earlier. They reported a marked elevation of both blood glucose (up to 2.5 $mmol \cdot L^{-1}$ above control levels) and plasma insulin levels (up to 3.5 times the values observed in the control trial)—accompanied by a 30% reduction in muscle

glycogenolysis when cycling at intensities varying between 45% and 75% VO_2max. These observed physiological changes are likely a combination of the lower exercise intensity and the intermittent nature of our extended work-bout design.

Paragraph 39: It has been reported that varied-intensity exercise or even short breaks in muscle work will allow for significant glucose uptake and possible short-term glycogen resynthesis (11, 16). In addition, contractile activity and insulin act synergistically to increase glucose uptake by the working muscle (29). It has also been estimated that the majority (96-100%) of glucose taken up by the muscle is oxidized during exercise (13). Jeukendrup et al. (14) has suggested that the complete suppression of hepatic glucose production (glucose Ra), in response to aggressive exogenous glucose ingestion, is a function of elevated insulin concentrations rather than high plasma glucose concentration. Despite complete suppression of hepatic glycogenolysis with large glucose feedings (22% solution; ~ 180 $g \cdot hr^{-1}$) during cycling at 50% VO_2max for 120 minutes, Jeukendrup et al. (14) did not demonstrate a reduction in the estimated rate of muscle glycogenolysis (estimated as the difference between total CHO oxidation and plasma glucose oxidation). This is in contrast to our findings in which a high insulin response concomitantly occurred with a reduction in muscle glycogenolysis, which was directly measured via biopsy. As previously mentioned, hepatic glycogenolysis cannot be determined from our study, but the relationship between the glucose, insulin, and muscle glycogen responses suggests that low intensity exercise (50% VO_2max) with periodic rest periods results in both higher blood glucose and insulin levels and sparing of muscle glycogen via increased exogenous CHO uptake and oxidation. The discrepancy between the current

study and that of Jeukendrup et al. (14) is likely a function of the exercise duration and the short rest periods between exercise segments.

Paragraph 40: Despite significantly higher blood glucose concentrations for much of the CHO trial compared to the control trial, Hargreaves et al. (12) found no difference in the circulating insulin concentrations and muscle glycogen utilization between PLA vs. CHO feedings when subjects were provided with 30 g CHO every 30 minutes, during 90 minutes of cycling at 70% VO_2max. Although this feeding schedule (60 $g \cdot hr^{-1}$) is the same as the present investigation, it is possible that the higher exercise intensity suppressed insulin release and reduced exogenous CHO uptake, thereby limiting glycogen sparing. Additionally, the absence of rest periods in Hargreaves et al.'s study possibly increased glycogenolysis or did not promote glycogen resynthesis.

Paragraph 41: Previous research has primarily examined the role of muscle glycogen during exercise of higher intensity when there are likely a high number of muscle fibers being recruited and glycogen depletion is more likely (12, 20, 25, 26, 27). The current study indicates that glycogen depletion may be a source of muscle fatigue even at moderate intensities during extended, discontinuous work. Support for these findings is provided by a study by Febbraio and Dancey (7) who concluded that muscle glycogen availability was a limiting factor during prolonged exercise (~ 2.6 to 3.6 hours) performed below the lactate threshold. It has been suggested that during intermittent, lower-intensity activities CHO supplementation may allow for muscle glycogen resynthesis in non-active type II fibers (25, 26) or that reduced muscle glycogenolysis is

primarily limited to type I fibers (25, 26, 30). Interestingly, the current protocol included several higher-intensity intervals during each cycle and treadmill exercise segment, resulting in a diverse recruitment of fiber types. It is possible that the five minutes of rest every 30 minutes and/or the incremental intensities may have contributed to the preservation or resynthesis of glycogen during the CHO trial.

Paragraph 42: If glycogen depletion is a primary contributor to muscle fatigue, then a reduced rate of glycogenolysis could positively affect work output at the end of any physically challenging event. This may be particularly applicable to improving performance in ultra-endurance competitions or physically demanding occupations such as wildland firefighting and certain military operations. Evidence from our lab also suggests that long-duration workers (wildland firefighters) have improved reaction time and improved performance in some cognitive tasks when given exogenous CHO throughout the work shift when compared to a placebo.

Paragraph 43: Regardless of how carbohydrate feedings are eliciting their fatigue-delaying effects: via decreasing the rate of muscle glycogenolysis, maintaining euglycemia, or attenuating the onset of some other fatigue-causing factor, it is apparent from this study that exogenous carbohydrate provided at a rate of 60 $g \cdot hr^{-1}$ can significantly alter muscle substrate oxidation during prolonged, intermittent activity of varied intensity. The maintenance of total CHO oxidation rates and blood glucose levels during both cycle and treadmill exercise (with a slight, although significant drop during the final treadmill session) in conjunction with decreased rates of muscle glycogenolysis

is an important metabolic finding. These results suggest that exogenous CHO may be able to prevent hypoglycemia while also providing enough supplemental substrate to maintain whole-body CHO oxidation and reduce glycogenolysis even during exercise lasting as long as 8 hours. This study establishes links between the metabolic demands of shorter duration exercise and extended muscle work. Further research remains to be conducted to evaluate the effects of exogenous CHO feedings during ultra-endurance activity on glucose kinetics and fiber-specific muscle glycogenolysis. Additionally, while we attempted to model the demands on wildland firefighters or working military personnel, these data and the majority of the other data regarding the effectiveness of exogenous CHO during longer work bouts have been collected in the laboratory. It is suggested that future studies attempt to define the effects of CHO supplementation on muscle function during ultra-endurance work under field conditions.

Paragraph 44: In conclusion, the significant increase in blood glucose concentrations, in combination with the decreased rate of muscle glycogenolysis and the maintenance of whole-body CHO oxidation (cycle), suggests that perhaps a feeding strategy which provides at least 60 g CHO·hr^{-1} may not only prevent hypoglycemia but also provide enough supplemental CHO to sustain total CHO oxidation with less reliance on muscle glycogen stores during intermittent, varied-intensity exercise lasting as long as 8 hours.

ACKNOWLEDGMENTS

This research project was funded by the U.S. Air Force Special Operations Command (AFSOC).

CONFLICT OF INTEREST

The researchers in this study collaborated with Hyperion Biotechnologies, Inc.

The results of this study do not constitute endorsement of any particular carbohydrate food by the authors or ACSM.

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TABLES AND FIGURES

Captions to Figures:

Figure 1. Exercise protocol—intensity breakdown of 25 minutes on each mode.

Figure 2. Plasma lactate concentration (mmol · L^{-1}). Values are means ± SD. * $p<0.05$ average [lactate] CHO vs. average [lactate] PLA; † $p<0.05$ post vs. pre.

Figure 3. Plasma glucose concentration (mmol · L^{-1}). Values are means ± SD. * $p<0.05$ CHO vs. PLA for the corresponding hour; † $p<0.05$ time point vs. pre (within trial).

Figure 4. Plasma insulin concentration (μIU · mL^{-1}). Values are means ± SD. * $p<0.05$ CHO vs. PLA for the corresponding hour; † $p<0.05$ time point vs. pre (within trial).

Figure 5a. Whole-body CHO oxidation (g · min^{-1}) on cycle ergometer. Values are means ± SD. * $p<0.05$ CHO vs. PLA for corresponding hour; † $p<0.05$ time point vs. hour 1 (within trial).

Figure 5b. Whole-body CHO oxidation (g · min^{-1}) on treadmill. Values are means ± SD. * $p<0.05$ vs. PLA for corresponding hour; † $p<0.05$ time point vs. hour 1 (within trial).

Figure 6a. Whole-body fat oxidation (g · min^{-1}) on cycle ergometer. Values are means ± SD. * $p<0.05$ vs. PLA for corresponding hour; † $p<0.05$ time point vs. hour 1 (within trial).

Figure 6b. Whole-body fat oxidation (g · min^{-1}) on treadmill. Values are means ± SD. * $p<0.05$ vs. PLA for corresponding hour; † $p<0.05$ time point vs. hour 1 (within trial).

Figure 7. Glycogen concentration (mmol · kg wet $wt.^{-1}$). Values are means ± SD. Bars indicate where there is a significant difference; $p<0.05$.

Table 1. *Descriptive characteristics of subjects*

	Males (n = 8)
Age, yr	28.9 ± 11.5
Height, cm	177.8 ± 5.3
Body mass, kg	74.0 ± 11.6
Body fat, %	9.1 ± 5.7
Fat-free mass, kg	66.8 ± 7.6
Cycle ergometer	
VO_2max	
$L \cdot min^{-1}$	4.2 ± 0.6
$ml \cdot kg^{-1} \cdot min^{-1}$	57.4 ± 7.6
$\%VO_2max$ @ Tvent	66.0 ± 0.1
Treadmill	
VO_2max	
$L \cdot min^{-1}$	4.6 ± 0.5
$ml \cdot kg^{-1} \cdot min^{-1}$	62.4 ± 7.4
$\%VO_2max$ @ Tvent	62.6 ± 0.1

Values are means ± SD. VO_2max = maximal O_2 uptake; Tvent = ventilatory threshold.

Table 2. *Average prescribed workloads for exercise trials*

	70% T_{vent}	90% T_{vent}	100% T_{vent}	110% T_{vent}
Cycle ergometer (watts)	143 ± 11.0	199 ± 11.8	227 ± 15.4	255 ± 19.8
Treadmill (percent grade)	9 ± 0.9	13 ± 1.2	16 ± 1.4	18 ± 1.4

Values are means ± SD. Tvent = ventilatory threshold. Treadmill speed was constant at 106.8 m · min^{-1}.

Table 3a. *Heart rate during cycle ergometer segments*

		Time (Hours)			
		1	4	5	8
CHO					
	70% Tvent	132 ± 9	133 ± 12	138 ± 13	142 ± 9
	110% Tvent	150 ± 9	155 ± 9	155 ± 10	153 ± 9
PLA					
	70% Tvent	132 ± 10	133 ± 12	141 ± 12	141 ± 10
	110% Tvent	151 ± 10	152 ± 13	157 ± 9	154 ± 9
Time x Intensity					
	70% Tvent	132 ± 10	133 ± 12	138 ±12*	141 ±10*
	110% Tvent	151 ±10	154 ±13†	156 ± 9†	153 ± 10†

Values are means ± SD. Units are beats per minute. * $p<0.05$ vs. hour 1 70% Tvent; † $p<0.05$ vs. hour 1 110% Tvent.

Table 3b. *Heart rate during treadmill segments*

		Time (Hours)			
		1	4	5	8
CHO					
	70% Tvent	126 ± 12	130 ± 9	137 ± 14	137 ± 16
	110% Tvent	153 ± 9	154 ± 9	155 ± 10	151 ± 8
PLA					
	70% Tvent	128 ± 10	133 ± 12	139 ± 13	135 ± 12
	110% Tvent	149 ± 11	154 ± 13	158 ± 12	155 ± 9
Time					
		138 ± 16	142 ± 16*	146 ± 15*	143 ±14*

Values are means ± SD. Units are beats per minute. * $p<0.05$ vs. hour 1 (main effect for time).

Table 4a. *RPE during cycle ergometer segments*

	Time (Hours)			
	1	4	5	8
CHO				
70% Tvent	10.5 ± 0.8	11.3 ± 0.9	11.3 ± 0.9	13.0 ± 2.3
110% Tvent	12.9 ± 0.8	13.9 ± 1.1	14.1 ± 0.6	15.9 ± 2.0
PLA				
70% Tvent	11.0 ± 1.1	11.5 ± 0.9	11.5 ± 0.9	14.8 ± 2.7
110% Tvent	13.0 ± 0.9	14.3 ± 0.9	14.6 ± 1.4	16.0 ± 2.3
Time x Intensity				
70% Tvent	10.7 ± 0.9	11.4 ± 0.9	11.4 ± 0.9	13.8 ± 2.0*
110% Tvent	12.9 ± 0.9	14.1 ± 1.0†	14.4 ± 1.1†	15.9 ± 2.6†

Values are means ± SD. * $p<0.05$ vs. hour 1 70% Tvent; † $p<0.05$ vs. hour 1 110% Tvent.

Table 4b. *RPE during treadmill segments*

	Time (Hours)			
	1	4	5	8
CHO				
70% Tvent	10.6 ± 0.7	11.3 ± 0.7	11.6 ± 1.1	12.5 ± 2.8
110% Tvent	13.0 ± 0.8	13.9 ± 1.1	14.0 ± 1.1	15.0 ± 2.6
PLA				
70% Tvent	11.3 ± 0.5	11.1 ± 1.0	11.9 ± 0.8	13.7 ± 2.9
110% Tvent	13.5 ± 0.9	14.3 ± 1.7	14.9 ± 1.6	16.1 ± 2.8
Time				
	12.1 ± 1.4	12.5 ± 1.7	12.9 ± 1.6	14.0 ± 2.9*

Values are means ± SD. * $p<0.05$ vs. hour 1 (main effect for time).

Figure 1

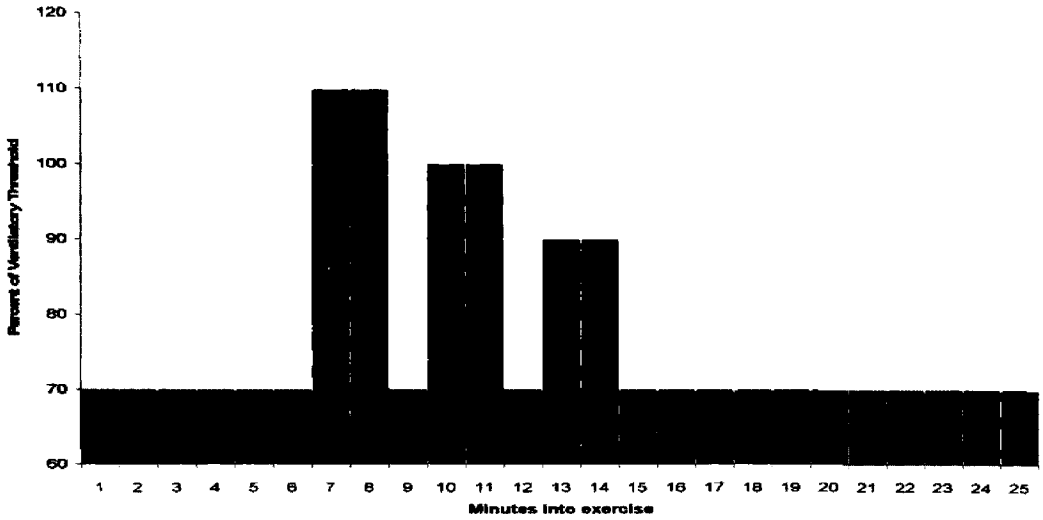


Figure 2

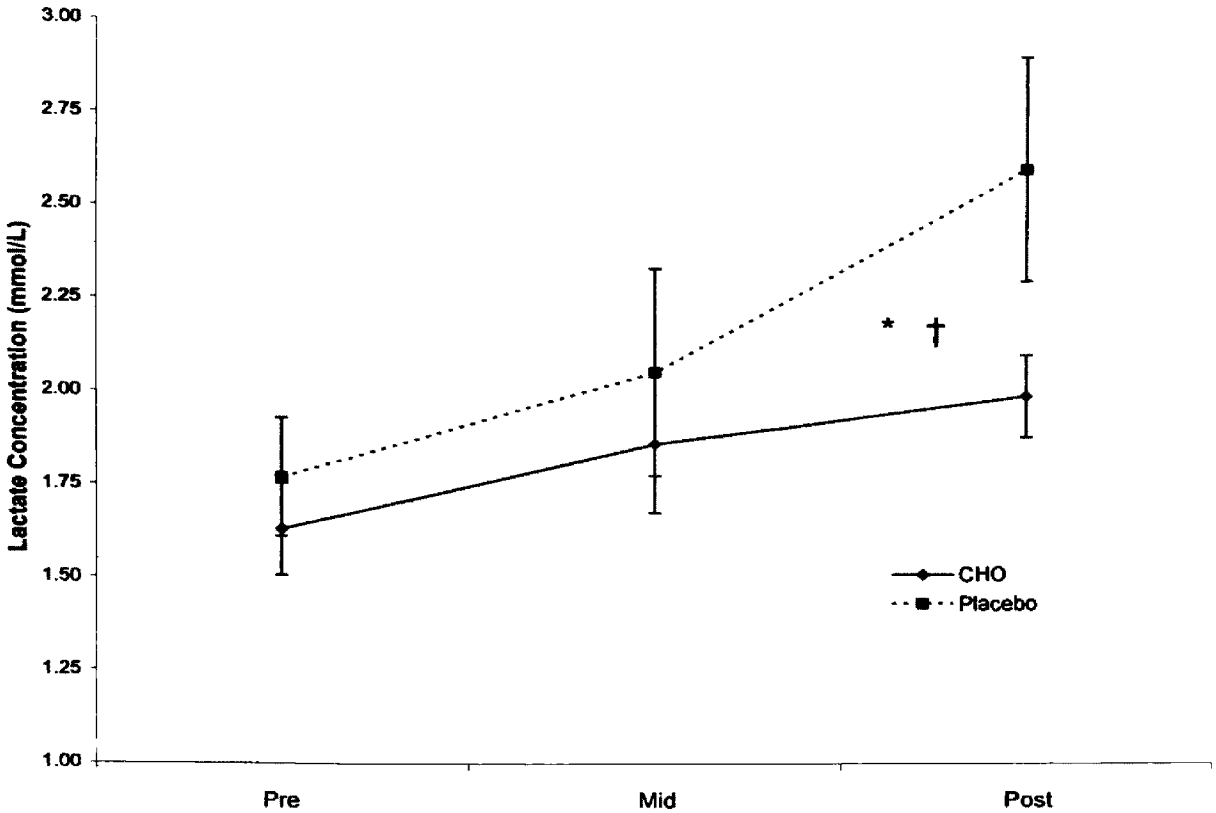


Figure 3

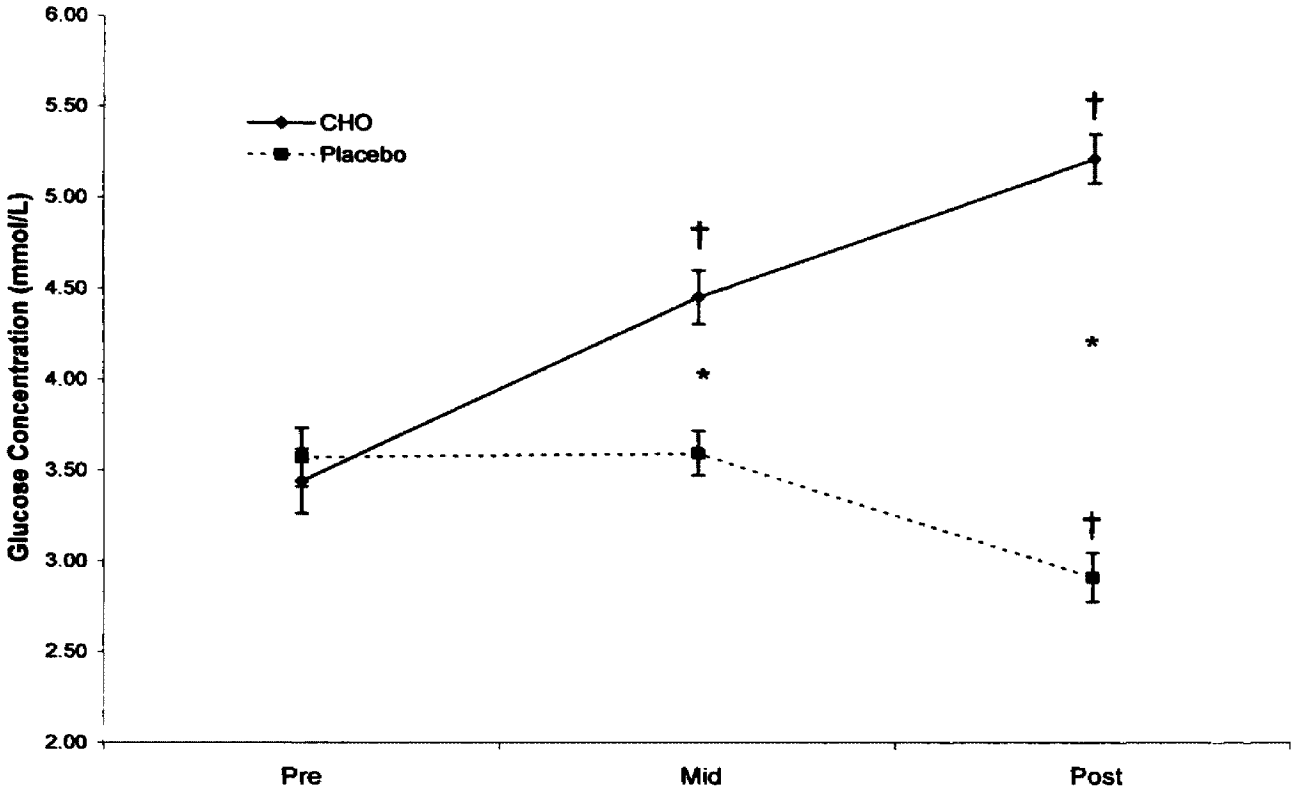


Figure 4

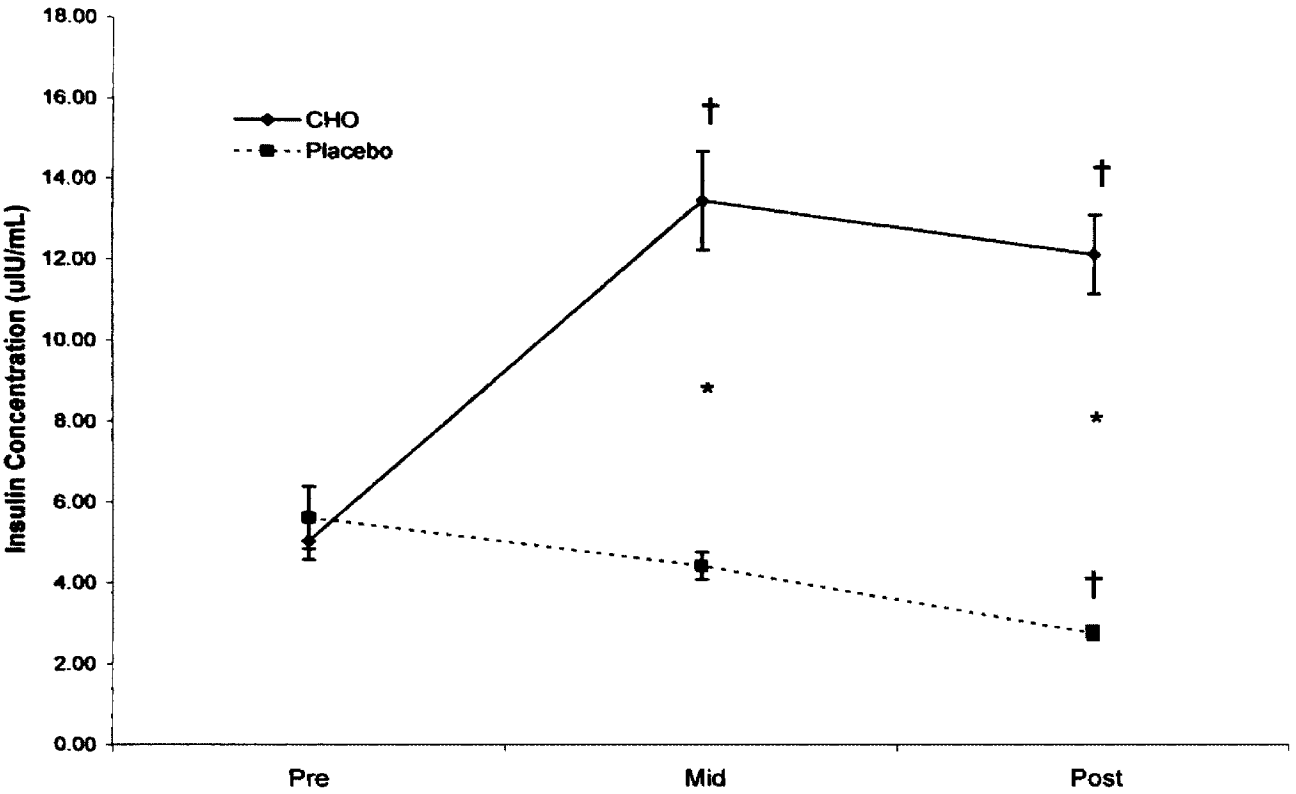


Figure 5a

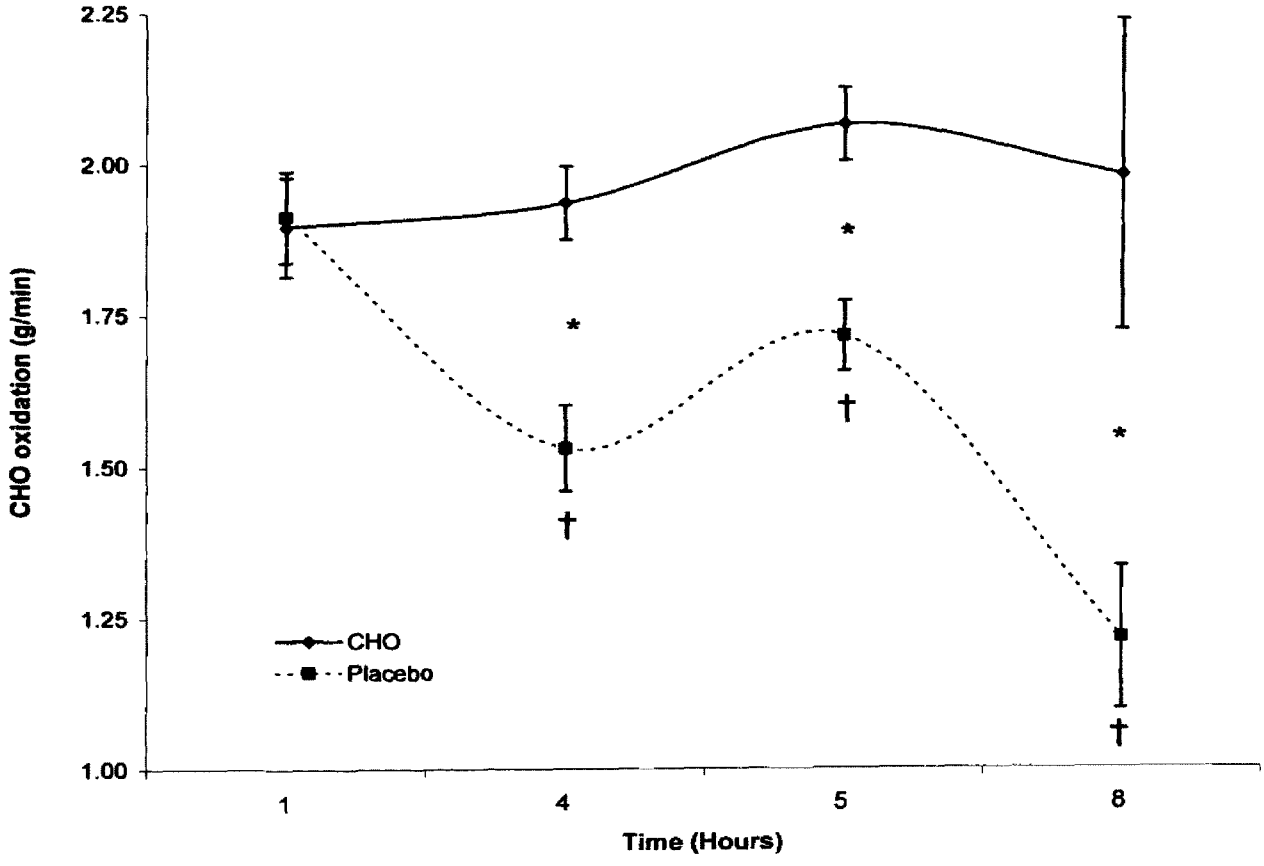


Figure 5b

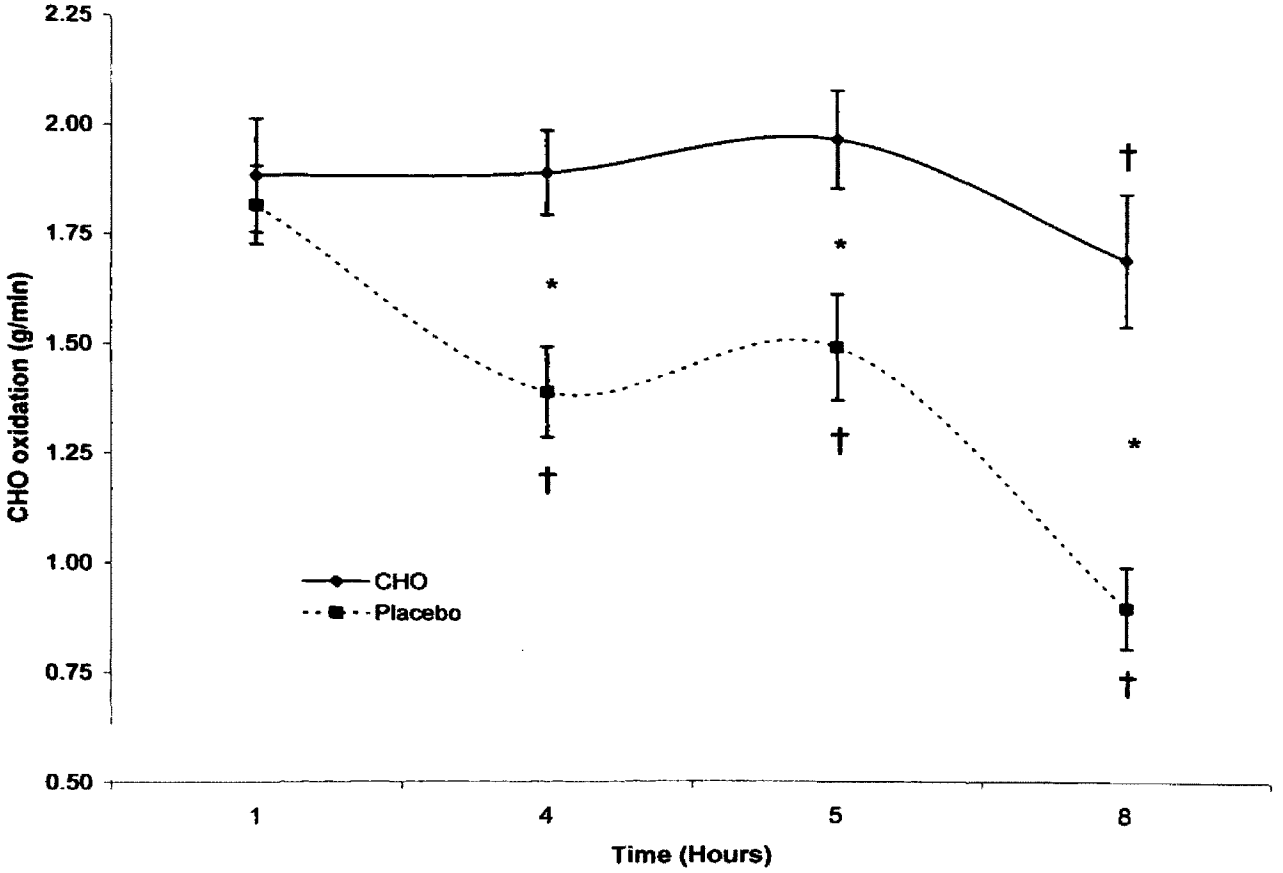


Figure 6a

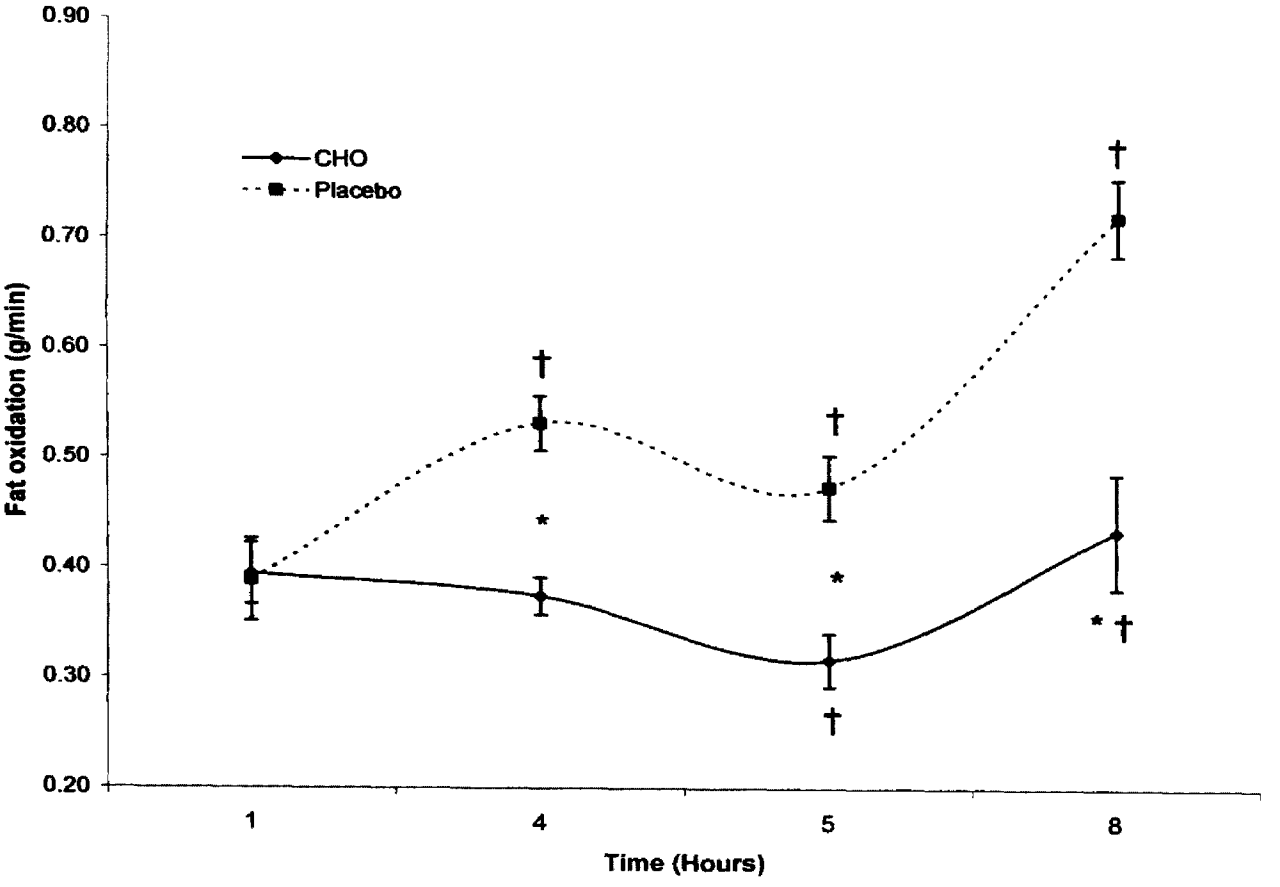


Figure 6b

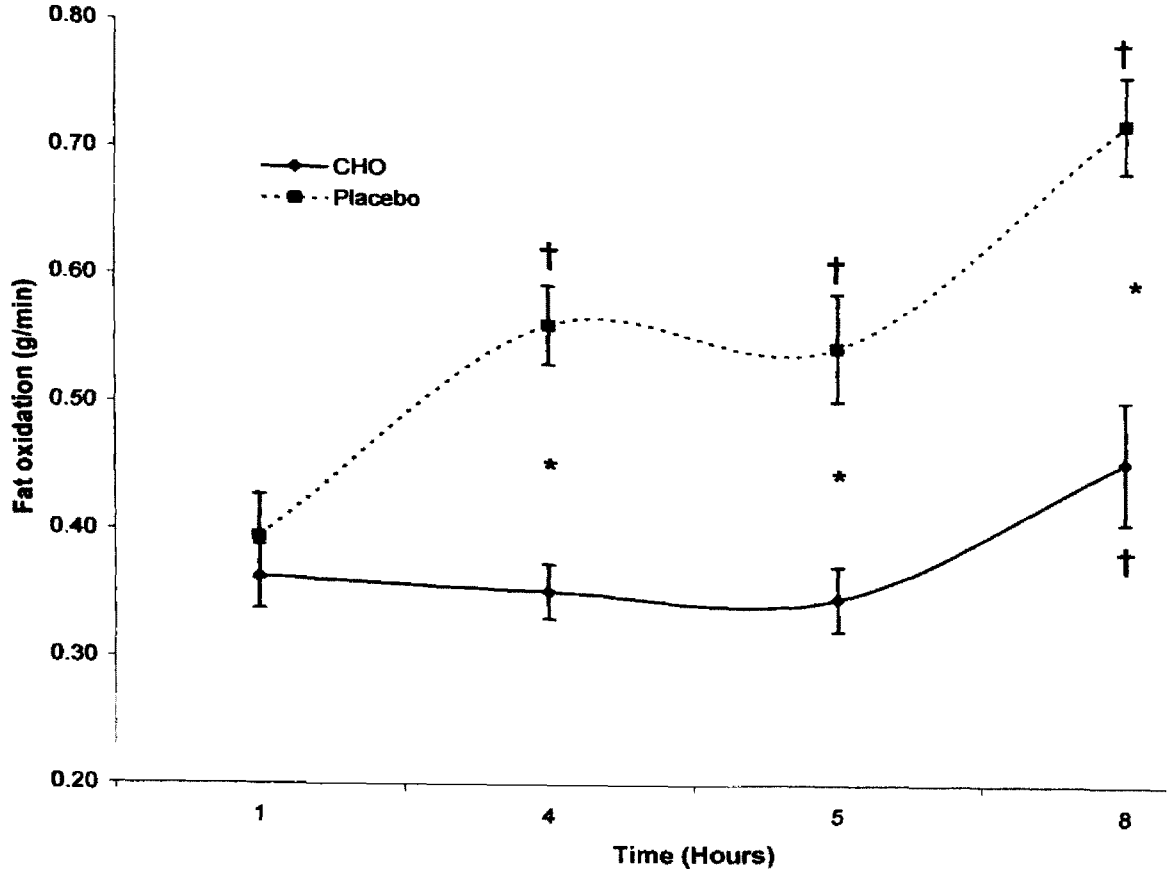
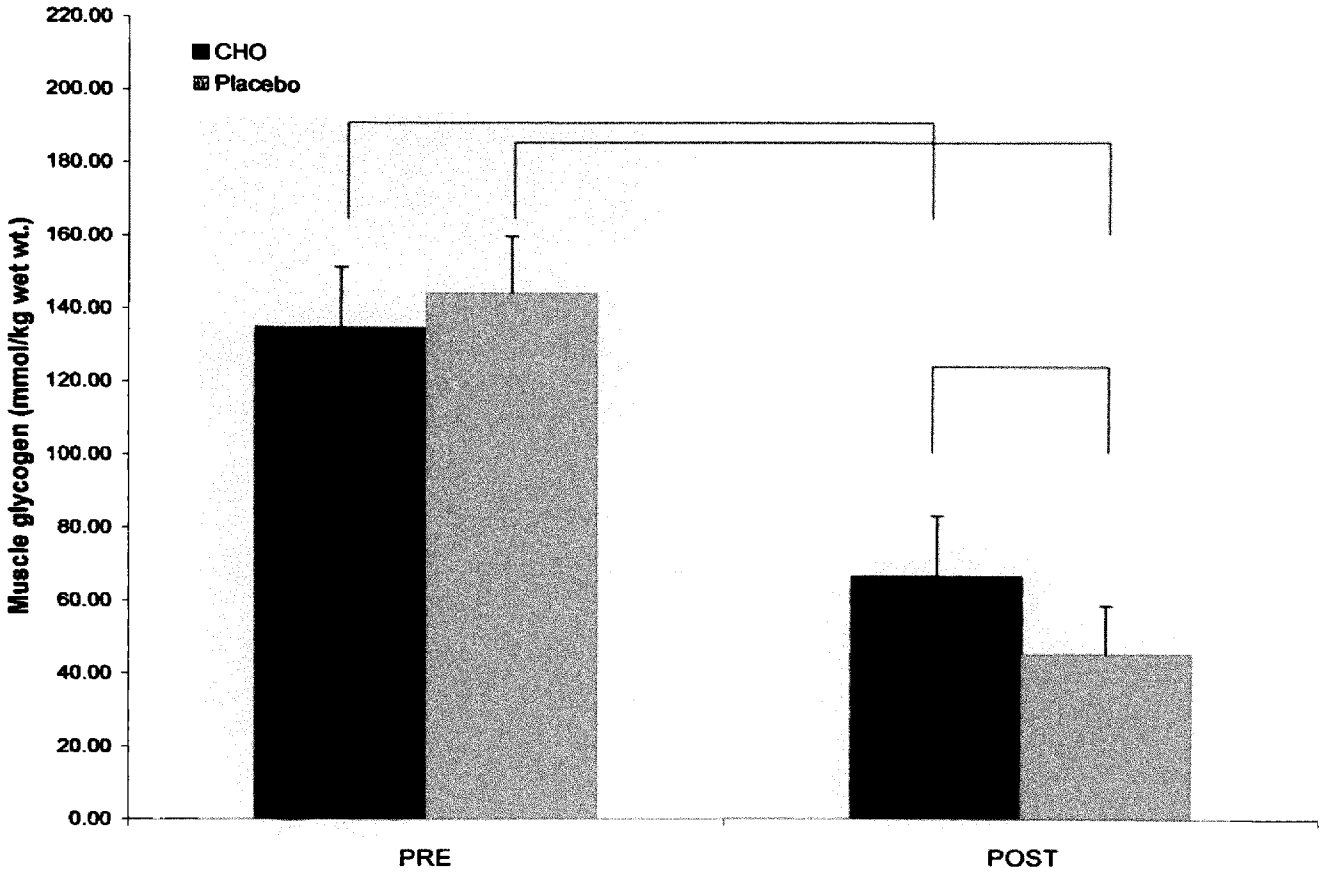


Figure 7



APPENDICES

Informed Consent Form

Effects of carbohydrate feedings on blood glucose and muscle glycogen during extended exercise

Principal Investigator: **Brent C. Ruby, Ph.D.**

Location: **Human Performance Laboratory**
McGill Hall #121
The University of Montana
Missoula, MT 59812
(406) 243-2117/(406) 243-4780

Purpose
The purpose of this project is to determine the effects of high carbohydrate food/drink sources on blood glucose, and changes in muscle glycogen during extended exercise. The information collected in this study will help determine if the effects of supplemental carbohydrate consumption will alter work performance and minimize fatigue during extended exercise/work bouts.

As a participant in this study you will complete the following assessments. 1) a pre-screening assessment which involves a health/exercise history questionnaire (Par-Q), 2) a measure of percent body fat obtained using a underwater weighing, 3) a maximal treadmill test and maximal cycle ergometer test to measure aerobic fitness levels, 4) two eight-hour work/rest sessions involving cycling and treadmill walking/jogging 5) the completion of a mood state survey (the Profile of Mood States) at six times during the study, 5) collection of expired gases to determine fuel use during the 8-hour work/rest session, 6) consumption of one of two different types of liquid carbohydrate every hour of exercise, 7) venous blood samples collected from an arm vein (pre-exercise, after 4 and 7 hours and immediately post-exercise) for measures of blood glucose, lactate, and epinephrine, 8) measures of cognitive performance using a computer reaction time, verbal, and math test, 9) saliva samples collected at various times before, during and after each exercise session, 10) a total of four muscle biopsies (two muscle biopsies per 8-hr trial: a) early in the morning before the exercise session begins, and b) late in the afternoon after the exercise session is completed),

Body Fat Measurement – Underwater Weighing:
This test session will require that you do not eat for a minimal of 3 hours prior to the testing. Prior to the test, body weight will be recorded in your bathing suit. You will then be asked to complete between 3 – 6 underwater weighing procedures. The underwater weight requires that you are submersed in our weighting tank (similar to a hot tub) and that you maximally exhale as much air as possible while underwater. The underwater weight will be recorded within 2-4 seconds and then you will be signaled to surface. This procedure will be repeated until three measurements have been obtained that are within 100 grams of each other. A nose clip will be provided upon request. This test will take approximately 30 minutes.

Maximal Exercise Test – Treadmill:
This test will consist of walking and running on a motorized treadmill to a maximal effort. The speed and grade of the treadmill will progress to fatigue. You will be encouraged to continue to walk/run until exhaustion. During the entire testing session on the treadmill, you will wear a nose clip and headgear that will support a mouthpiece. This will allow us to measure the amount of oxygen the body uses during the exercise. Heart rate will be measured using an elastic chest strap that is worn on the skin under your shirt around your chest. This test will take approximately 45 minutes to 1-hour. You will be asked to fast for approximately 3 hours prior to this test.

Maximal Exercise Test – Cycle:
This test will consist of riding on a laboratory exercise cycle to maximal effort. The resistance of the cycle will increase each minute and will progress to fatigue. You will be encouraged to continue to ride until exhaustion. During this test you will wear a nose clip and

headgear that will support a mouthpiece. This will allow us to measure the amount of oxygen that the body uses during this exercise. Heart rate will be measured using an elastic chest strap that is worn on the skin under your shirt around your chest. This test will take approximately 45 minutes to 1 hour. You will be asked to fast for approximately three hours prior to this test.

8-hour Exercise/Rest Session:

You will be asked to report to the laboratory after a 12 hour fast, at approximately 6:00 AM. Upon arrival, you will be asked to void your bladder and your nude body weight will be measured. A cognitive function test using a computer program will evaluate your decision-making skills and reaction times. A saliva sample will be collected using a cotton swab that is placed in your mouth near your cheek for approximately 5 minutes. Prior to exercise, a blood samples will be obtained from an arm vein (7-10 ml). A muscle biopsy will then be obtained from your front thigh muscle (this is detailed below). You will then be provided with a small, standardized breakfast (apple, granola bar, juice box). Each of the 8-hr sessions will be completed as eight, 1-hour sessions as follows:

1) **Twenty-five minutes of cycling.** This exercise period will be initiated with six minutes of cycling at 40-50% of your maximal capacity followed by a sequence of intervals that includes three, two minute cycling intervals completed at approximately 70-75% of your maximal capacity. Each of these intervals will be followed by a 1-minute active recovery period, cycling at 40-50% of your maximal capacity. This series of intervals will then be followed by a remaining 10 minutes of cycling at 40-50% of your maximal capacity.

2) **Five-minute rest** and transition to treadmill.

3) **Twenty-five minutes of treadmill exercise.** This exercise period will be initiated with six minutes of treadmill walking at 40-50% of your maximal capacity followed by a sequence of intervals that includes three, two minute treadmill jog/run intervals completed at approximately 70-75% of your maximal capacity. Each of these intervals will be followed by a 1-minute active recovery period, walking at 40-50% of your maximal capacity. This series of intervals will then be followed by a remaining 10 minutes of walking at 40-50% of your maximal capacity.

4) **Five-minute rest.**

Additional saliva samples will be collected after each hour of exercise. After the completion of the fourth 1-hr session, a second blood sample will be obtained and you will be asked to complete the computer cognitive performance test and provide a saliva sample. You will then be given a small snack lunch and an additional 20 minutes of rest. The remaining four 1-hr sessions will be identical to those above. A third blood sample will be obtained after the 7th hour of exercise. After the final 8th exercise period, a fourth blood sample and the final saliva sample will be obtained. This will be followed by a post exercise nude body measurement and the post-exercise muscle biopsy. Following the completion of the muscle biopsy, you will be provided with a meal in the lab.

Muscle Biopsies:

A total of four muscle biopsies will be obtained from your front thigh muscle (vastus lateralis, approximately 6 inches up from the kneecap on the lateral side of your thigh). The muscle biopsy procedure requires that the site be sterilized. After the site is cleaned, a small amount of lidocaine will be injected just under the skin surface. Additional small amounts of lidocaine will be injected around a small 1-inch area around the site on the leg. After the area is treated with the lidocaine (approximately 5 ml, 1% lidocaine), a small incision (approximately 1/4 inch long) will be made through the skin and to a depth of approximately 3/4-1.5 inches. The biopsy needle will then be inserted through the incision and the sample obtained. After the sample is obtained, the site will be cleaned and closed with steri-strips

and/or a single stitch and bandaid and wrapped with an ace bandage. A flexible ice pack will then be placed on the site for 10 minutes. The biopsy samples will be obtained a) prior to the 8-hour exercise/rest session, and b) immediately after the 8-hour exercise/rest session (on the same leg but approximately 2 inches above the initial sample). This will be repeated for the second trial using the opposite leg. The muscle biopsies will be used to evaluate alterations in muscle carbohydrate and fat stores in response to physical activity. Latex free bandages will be provided if subjects have a known allergy to latex. All of the muscle biopsies will be conducted by Dr. Brent Ruby, the study director.

Body Weight:

Your nude body weight will be measured using a digital scale prior to, after 4-hours, and immediately following each 8-hour exercise/rest session. All measures will be done in private.

Surveys and Cognitive Testing:

You will be asked to complete a 60-question survey that has been developed to assess you opinions regarding your current mental state prior to, at the mid point and immediately following the each 8-hour exercise/rest session. You are asked to provide an accurate and truthful response to each question. You will also complete a computer directed test that will measure reaction time and accuracy to a series of questions.

Supplemental Carbohydrate Beverage:

During each 8-hour exercise/rest session, you will be given one of two different carbohydrate-containing beverages. You will be asked to drink a predetermined amount (approximately 100 ml, approximately 3.4 oz, approximately 1/2 cup) of the solution at the top of each hour and 30 minutes into each of the eight-1hr exercise periods. You will also be provided with a small, standardized lunch at the 4-hour midpoint.

Arm vein blood samples :

During each 8-hour exercise session, periodic blood samples will be collected (pre-exercise, 4 hour, 7 hour, post-exercise). These samples are being collected to evaluate changes in blood sugar throughout each exercise session as well as lactate and epinephrine. All of the blood samples will be obtained by Dr. Brent Ruby, the study director and trained phlebotomist.

Risks and Discomfort

1. Mild discomfort may result during and after the exercise. These discomforts include shortness of breath, tired or sore legs, nausea and possibility of vomiting,
2. Muscle soreness after the tests may occur as a result of the exercise, but should not persist.
3. Certain changes in body function take place when any person exercises. Some of these changes are normal and others are abnormal. Abnormal changes may occur in blood pressures, heart rate, heart rhythm or extreme shortness of breath. Very rare instances of heart attack have occurred. Every effort will be made to minimize possible problems by the preliminary evaluation and constant surveillance during testing. A trained CPR technician will be on hand at all times and the laboratory has standard emergency procedures should any potential problems arise.
4. You will be informed of any new findings that may affect your decision to remain in the study.
5. The muscle biopsy and blood sampling techniques may cause some local and temporary discomfort. It is normal to have the sensation of a deep tissue bruise around the site of the muscle biopsy. This pain should be manageable and not above the pain associated from a "charlie horse" type bruise.
6. There is a minor risk of infection associated with blood sampling and the muscle biopsy. Should you notice unusual redness, swelling or drainage at the biopsy incision site or at the sites of the blood sampling sites you should seek medical attention and then notify Brent Ruby, study director.
7. There are minimal risks associated with the use of lidocaine (the local anesthetic). The risk of a reaction to the lidocaine is extremely low (approximately 1/1,000,000). However to minimize this risk, no more than 5-9 ml of a 1% lidocaine solution will be used per biopsy.
8. During any of the exercise tests should symptoms, such as chest discomfort, unusual shortness of breath or other abnormal findings develop, the exercise physiologist conducting the research will

terminate the test. Guidelines by the American College of Sports Medicine will be followed to determine when a test should be stopped. These symptoms include moderate to severe angina (chest pain), increased dizziness, shortness of breath, fatigue and your desire to stop.

9. You will be excluded from participation if you have a known history of allergic reactions to local anesthetics.

Benefits of Participating in This Study

1. Upon completion of the preliminary tests (body fat, treadmill, cycle and arm ergometer max tests), you will be paid $25. Upon completion of the first 8-hour exercise/rest session, you will be paid another $75. Upon completion of the second 8-hour session, you will be paid another $100. Therefore, upon completion of the entire study, you will be paid a total of $200. If you decide to withdraw at any time, you will be compensated for the test sessions you have completed or initiated.

2. The information from these tests will provide you with an accurate assessment of you aerobic fitness and body composition that can be compared with norms for your age and sport but may be of little benefit to your understanding of your personal fitness. There are no other direct benefits to the participants in the study.

3. There is no promise that you will receive any benefit outside of the financial payment as a result of taking part in this study.

Confidentiality

All results will be kept in strict confidence among the subject involved and the Principal Investigators and other Co-Investigators. During the entire period of data collection, subject records will be kept within the Human Performance Laboratory and will be locked under the direction of the Principal Investigator.

Compensation for Injury

Although we believe that the risk of taking part in this study is minimal, the following liability statement is required in all University of Montana consent forms. *In the event that you are injured as a result of this research you should individually seek appropriate medical treatment. If the injury is caused by negligence of the University or any of its employees, you may be entitled to reimbursement pursuant to the Comprehensive State Insurance Plan established by the Department of Administration under the authority of M.C.A., Title2, Chapter 9. In the event of a claim for such injury, further information may be obtained from the University's Claim representative or University Legal Counsel.*

Voluntary Participation and Withdrawl

It is important that you realize that you are free to withdraw from the study at any time. As mentioned above, even if you decide to drop out of the study, you will receive full compensation for all the test sessions you complete or initiate.

A copy of this consent form will be provided for you at your request. In addition, the data collected during this study will be done at no cost to you.

Statement of Consent

I have read the above statements and understand the risks involved with this study. I authorize Brent C. Ruby and such assistants that he may designate, to administer and conduct the testing as safely as possible with a minimal amount of discomfort. If I have additional questions, I may contact Brent C. Ruby at home (406) 542-2513, cell (406) 546-4691 or at the Human Performance Laboratory (406) 243-2117.

Participant (print) _____

Signature _____ Date _____

Revised 11/2004

Permanent Mailing Address
(for payment purposes) _____

_____

_____

Investigator/Witness Signature
(print) _____

Investigator/Witness Signature _____ Date _____

Subject statement of consent to be photographed during data collection

During the study, I understand that pictures may be taken. I provide my consent to having my picture taken during the course of the research study. I provide my consent that my picture may be used in some presentations related to this study. If pictures are used at any time for presentation, names will not be associated with them.

Signature _____ Date _____

Approval Expires On 11/29/05

Date Approved by UM IRB 11-30-04

IRB Chair

Revised 11/2004

(Hour 1)

8:00 AM BEGIN EXERCISE (cycle)
*start main clock
*drink provided
8:15 AM drink provided / **calibrate**
8:18 AM begin gas collection
8:25 AM stop exercise
*begin 5 min rest
8:30 AM begin exercise (treadmill)
*drink provided
8:45 AM drink provided
8:48 AM begin gas collection
8:55 AM stop exercise
*jump test
*remainder of 5 min rest

(Hour 2)

6:15 AM subject arrives
6:30 AM *HRV
6:45 AM *blood draw
*biopsy
*saliva
7:15 AM subject eats breakfast
7:55 AM void bladder (toilet)
*body weight
*start HR monitor
7:59 AM jump test

(Hour 3)

10:00 AM begin exercise (cycle)
*drink provided
10:15 AM drink provided
10:25 AM stop exercise
*begin 5 min rest
10:30 AM begin exercise (treadmill)
*drink provided
10:45 AM drink provided
10:55 AM stop exercise
*jump test
*remainder of 5 min rest

(Hour 4)

9:00 AM begin exercise (cycle)
*drink provided
9:15 AM drink provided
9:25 AM stop exercise
*begin 5 min rest
9:30 AM begin exercise (treadmill)
*drink provided
9:45 AM drink provided
9:55 AM stop exercise
*jump test
*remainder of 5 min rest

11:00 AM begin exerc
*drink provi
11:15 AM drink provic
11:18 AM begin gas c
11:25 AM stop exerci
*begin 5 mi
11:30 AM begin exerc
*drink provi
11:45 AM drink provic
11:48 AM begin gas c
11:55 AM stop exerci
*jump test
*blood drav
*saliva
*lunch

(Hour 5)

12:30 PM BEGIN EXERCISE (cycle)
*restart main clock
*drink provided
12:45 PM drink provided / **calibrate**
12:48 PM begin gas collection
12:55 PM stop exercise
*begin 5 min rest
1:00 PM begin exercise (treadmill)
*drink provided
1:15 PM drink provided
1:18 PM begin gas collection
1:25 PM stop exercise
*jump test
*begin 5 min rest

(Hour 6)

1:30 PM begin exercise (cycle)
*drink provided
1:45 PM drink provided
1:55 PM stop exercise
*begin 5 min rest
2:00 PM begin exercise (treadmill)
*drink provided
2:15 PM drink provided
2:25 PM stop exercise
*jump test
*blood draw
*remainder of 5 min rest

(Hour 7)

2:30 PM begin exercise (cycle)
*drink provided
2:45 PM drink provided
2:55 PM stop exercise
*begin 5 min rest
3:00 PM begin exercise (treadmill)
*drink provided
3:15 PM drink provided
3:25 PM stop exercise
*jump test
*remainder of 5 min rest

(Hour 8)

3:30 PM begin exercise (cycle)
*drink provided
3:45 PM drink provided / **calibrate**
3:48 PM begin gas collection
3:55 PM stop exercise
*begin 5 min rest
4:00 PM begin exercise (treadmill)
*drink provided
***call / order food**
4:15 PM drink provided
4:18 PM begin gas collection
4:25 PM stop exercise
*jump test
*blood draw
*biopsy
*saliva
*HRV
*collect final urine in bucket
EAT DINNER!!!!!

8 HOUR STUDY -- Jamie Wagner

Baseline Testing	Baseline Testing	Baseline Testing	Baseline Testing	Baseline Testing	Baseline Testing	Baseline Testing	Baseline Testing
Subject #	Age	Height (cm)	Weight (kg)	% Body Fat	Lean Body Mass (kg)	Cyc VO2max (ml/kg/min)	Cyc VO2max (L/min)
1	48	175.3	84.3	17.8	69.3	42.96	3.57
2	19	177.8	62.4	3.1	60.5	67.63	4.22
3	21	182.9	79.5	4.8	75.7	58.39	4.64
4	23	175.3	67.3	4.5	64.3	54.63	3.68
5	28	182.9	89.4	14.0	76.9	55.64	4.97
7	25	177.8	64.7	9.0	58.9	55.31	3.72
8	46	182.9	84.0	14.7	71.6	58.67	4.92
9	21	167.6	60.0	4.5	57.3	65.80	3.95
Average	**28.9**	**177.8**	**74.0**	**9.1**	**66.8**	**57.38**	**4.21**
STD	**11.53**	**5.27**	**11.56**	**5.71**	**7.64**	**7.57**	**0.57**
Trial A	Trial A	Trial A	Trial A	Trial A	Trial A	Trial A	Trial A
Subject #	Trial	HR 1st Int Cyc 1	HR 2nd Int Cyc 1	HR 3rd Int Cyc 1	HR Last 5 Min Cyc 1	HR 1st Int Cyc 2	HR 2nd Int Cyc 2
1	A	145	145	144	133	153	148
2	A	153	149	148	137	154	158
3	A	153	151	144	128	148	145
4	A	161	157	157	134	148	155
5	A	153	153	147	134	157	149
7	A	148	146	144	134	155	149
8	A	131	129	125	112	138	134
9	A	159	160	162	143	166	168
Average	**A**	**150.38**	**148.75**	**146.38**	**131.88**	**152.38**	**150.75**
STD	**A**	**9.40**	**9.48**	**10.91**	**9.06**	**8.12**	**9.97**
SE	**A**	**3.32**	**3.35**	**3.86**	**3.20**	**2.87**	**3.52**
Trial B	Trial B	Trial B	Trial B	Trial B	Trial B	Trial B	Trial B
Subject #	Trial	HR 1st Int Cyc 1	HR 2nd Int Cyc 1	HR 3rd Int Cyc 1	HR Last 5 Min Cyc 1	HR 1st Int Cyc 2	HR 2nd Int Cyc 2
1	B	136	142	132	125	138	137
2	B	157	157	151	143	164	160
3	B	147	142	137	129	150	144
4	B	156	155	147	133	153	160
5	B	163	159	153	142	165	156
7	B	147	147	145	129	147	150
8	B	140	137	135	114	131	128
9	B	160	161	155	140	158	155
Average	**B**	**150.75**	**150.00**	**144.38**	**131.88**	**150.75**	**148.75**
STD	**B**	**9.74**	**9.12**	**8.73**	**9.83**	**11.97**	**11.57**
SE	**B**	**3.44**	**3.22**	**3.09**	**3.48**	**4.23**	**4.09**

Baseline Testing		Baseline Testing		Baseline Testing		Baseline Te
Cyc 70% VT (ml/kg/min)	Cyc Watts 70% VT	Cyc 90% VT (ml/kg/min)	Cyc Watts 90% VT	Cyc 100% VT (ml/kg/min)	Cyc Watts 100% VT	Cyc 110% VT (ml/kg/min)
23.14	157	29.75	202	33.06	225	36.37
30.78	160	39.57	203	43.97	225	48.37
23.66	140	30.42	194	33.80	221	37.18
26.43	132	33.98	187	37.75	215	41.53
22.79	150	29.3	212	32.56	243	35.82
25.57	135	32.88	189	36.53	216	40.18
28.76	139	36.98	218	41.09	258	45.20
29.52	133	37.95	187	42.17	215	46.39
26.33	**143**	**33.85**	**199**	**37.62**	**227**	**41.38**
3.08	**10.98**	**3.96**	**11.76**	**4.40**	**15.44**	**4.84**
Trial A	Trial A	Trial A	Trial A	Trial A	Trial A	Trial A
HR 3rd Int Cyc 2	HR Last 5 Min Cyc 2	HR 1st Int Cyc 3	HR 2nd Int Cyc 3	HR 3rd Int Cyc 3	HR Last 5 Min Cyc 3	HR 1st Int Cyc 4
144	132	155	152	147	136	157
151	142	158	157	158	146	159
136	XXXXX	XXXXX	XXXXX	XXXXX	XXXXX	XXXXX
141	125	148	145	139	121	153
145	134	155	153	148	133	157
145	135	150	155	151	137	160
127	111	137	131	126	108	136
162	147	163	165	163	144	165
143.88	**132.29**	**152.29**	**151.14**	**147.43**	**132.14**	**155.29**
10.26	**11.74**	**8.36**	**10.71**	**12.23**	**13.41**	**9.25**
3.63	**4.15**	**2.96**	**3.79**	**4.32**	**4.74**	**3.27**
Trial B	Trial B	Trial B	Trial B	Trial B	Trial B	Trial B
HR 3rd Int Cyc 2	HR Last 5 Min Cyc 2	HR 1st Int Cyc 3	HR 2nd Int Cyc 3	HR 3rd Int Cyc 3	HR Last 5 Min Cyc 3	HR 1st Int Cyc 4
139	120	144	141	131	119	146
157	148	159	158	156	144	161
141	122	144	140	138	125	150
144	128	148	149	146	126	147
151	134	164	159	154	140	172
137	128	149	139	139	126	151
110	122	124	131	130	112	129
137	135	154	146	138	137	163
139.50	**129.63**	**148.25**	**145.38**	**141.50**	**128.63**	**152.38**
13.86	**9.26**	**12.08**	**9.66**	**9.71**	**10.90**	**13.07**
4.90	**3.27**	**4.27**	**3.42**	**3.43**	**3.85**	**4.62**

sting		Baseline Testing			Baseline Testing	
Cyc Watts 110% VT	TM VO2max (ml/kg/min)	TM VO2max (L/min)	TM 70% VT (ml/kg/min)	TM % grade 70% VT	TM 90% VT (ml/kg/min)	TM % grade 90% VT
247	52.27	4.41	23.93	7	30.76	11
248	70.44	4.40	29.93	10	38.48	15
248	66.02	5.25	28.19	9	36.24	13
243	62.65	4.21	27.17	8	34.93	13
274	59.18	5.29	24.96	9	32.09	13
243	56.98	3.69	26.39	9	33.93	13
297	57.62	4.84	28.63	9	36.81	14
242	74.03	4.44	27.98	9	35.97	14
255	**62.40**	**4.57**	**27.15**	**9**	**34.90**	**13**
19.80	**7.35**	**0.54**	**1.98**	**0.89**	**2.55**	**1.16**
Trial A	Trial A	Trial A	Trial A	Trial A	Trial A	Trial A
HR 2nd Int Cyc 4	HR 3rd Int Cyc 4	HR Last 5 Min Cyc 4	HR 1st Int Cyc 5	HR 2nd Int Cyc 5	HR 3rd Int Cyc 5	HR Last 5 Min Cyc 5
157	151	136	158	156	154	139
166	162	151	163	166	164	154
XXXXX	XXXXX	134	XXXXX	XXXXX	XXXXX	146
155	150	123	151	146	136	119
149	155	131	156	161	152	142
154	150	139	159	156	153	144
133	126	111	135	132	132	118
166	159	140	165	165	157	141
154.29	**150.43**	**133.13**	**155.29**	**154.57**	**149.71**	**137.88**
11.28	**11.73**	**11.99**	**10.05**	**12.03**	**11.50**	**12.78**
3.99	**4.15**	**4.24**	**3.55**	**4.25**	**4.07**	**4.52**
Trial B	Trial B	Trial B	Trial B	Trial B	Trial B	Trial B
HR 2nd Int Cyc 4	HR 3rd Int Cyc 4	HR Last 5 Min Cyc 4	HR 1st Int Cyc 5	HR 2nd Int Cyc 5	HR 3rd Int Cyc 5	HR Last 5 Min Cyc 5
145	145	128	150	153	148	137
159	164	150	162	167	167	156
151	151	133	162	162	159	143
149	141	127	150	154	146	131
162	160	142	174	171	164	155
145	144	133	157	154	143	143
139	137	112	145	138	129	118
162	157	142	152	152	147	141
151.50	**149.88**	**133.38**	**156.50**	**156.38**	**150.38**	**140.50**
8.65	**9.69**	**11.66**	**9.29**	**10.27**	**12.44**	**12.38**
3.06	**3.42**	**4.12**	**3.28**	**3.63**	**4.40**	**4.38**

Baseline Testing			
TM 100% VT (ml/kg/min)	TM % grade 100% VT	TM 110% VT (ml/kg/min)	TM % grade 110% VT
34.18	13	37.60	15
42.75	18	47.03	20
40.27	16	44.30	18
38.81	16	42.69	18
35.66	15	39.23	18
37.70	15	41.47	17
40.90	16	44.99	18
39.97	16	43.97	18
38.8	**16**	**42.7**	**18**
2.83	**1.41**	**3.11**	**1.39**

Trial A	Trial A	Trial A	Trial A	Trial A	Trial A	Trial A	Trial A
HR 1st Int Cyc 6	HR 2nd Int Cyc 6	HR 3rd Int Cyc 6	HR Last 5 Min Cyc 6	HR 1st Int Cyc 7	HR 2nd Int Cyc 7	HR 3rd Int Cyc 7	HR Last 5 Min Cyc 7
164	166	158	140	160	157	151	140
164	167	163	149	164	158	152	143
XXXXX	XXXXX	XXXXX	XXXXX	XXXXX	XXXXX	XXXXX	XXXXX
150	149	145	125	149	156	155	128
162	155	151	145	164	165	155	146
158	156	156	141	158	152	151	142
138	135	130	113	136	134	128	112
159	160	153	141	158	157	152	142
156.43	**155.43**	**150.86**	**136.29**	**155.57**	**154.14**	**149.14**	**136.14**
9.45	**11.00**	**10.79**	**12.68**	**10.00**	**9.69**	**9.48**	**12.09**
3.34	**3.89**	**3.82**	**4.48**	**3.53**	**3.42**	**3.35**	**4.27**

Trial B	Trial B	Trial B	Trial B	Trial B	Trial B	Trial B	Trial B
HR 1st Int Cyc 6	HR 2nd Int Cyc 6	HR 3rd Int Cyc 6	HR Last 5 Min Cyc 6	HR 1st Int Cyc 7	HR 2nd Int Cyc 7	HR 3rd Int Cyc 7	HR Last 5 Min Cyc 7
153	154	152	135	152	156	149	135
167	170	165	157	164	157	154	153
158	158	153	138	156	158	161	146
150	150	142	126	150	150	147	129
175	171	166	153	171	171	162	147
155	152	149	140	153	153	152	137
139	135	123	117	139	130	132	114
150	151	142	139	157	152	149	133
155.88	**155.13**	**149.00**	**138.13**	**155.25**	**153.38**	**150.75**	**136.75**
11.06	**11.59**	**13.86**	**13.01**	**9.53**	**11.41**	**9.38**	**12.24**
3.91	**4.10**	**4.90**	**4.60**	**3.37**	**4.04**	**3.32**	**4.33**

Trial A	Trial A	Trial A	Trial A	Trial A	Trial A	Trial A	Trial A
HR 1st Int Cyc 8	HR 2nd Int Cyc 8	HR 3rd Int Cyc 8	HR Last 5 Min Cyc 8	HR 1st Int TM 1	HR 2nd Int TM 1	HR 3rd Int TM 1	HR Last 5 Min TM 1
152	159	155	147	144	140	133	119
150	136	136	134	156	154	145	126
XXXXX	XXXXX	XXXXX	155	163	157	152	137
156	157	152	129	152	148	146	106
166	160	159	151	158	154	147	134
153	152	148	142	158	156	152	139
136	136	131	139	135	130	125	113
158	157	150	141	156	157	156	134
153.00	**151.00**	**147.29**	**142.25**	**152.75**	**149.50**	**144.50**	**126.00**
9.15	**10.55**	**10.16**	**8.60**	**9.05**	**9.77**	**10.46**	**12.17**
3.23	**3.73**	**3.59**	**3.04**	**3.20**	**3.45**	**3.70**	**4.30**
Trial B	Trial B	Trial B	Trial B	Trial B	Trial B	Trial B	Trial B
HR 1st Int Cyc 8	HR 2nd Int Cyc 8	HR 3rd Int Cyc 8	HR Last 5 Min Cyc 8	HR 1st Int TM 1	HR 2nd Int TM 1	HR 3rd Int TM 1	HR Last 5 Min TM 1
156	159	158	146	141	135	128	117
159	146	143	158	157	157	152	133
156	155	154	132	160	157	149	131
148	145	146	130	151	152	146	130
170	165	163	150	166	165	164	146
144	153	151	143	144	148	142	128
141	139	135	129	133	126	122	114
156	155	155	136	140	133	130	125
153.75	**152.13**	**150.63**	**140.50**	**149.00**	**146.63**	**141.63**	**128.00**
9.24	**8.37**	**8.96**	**10.47**	**11.36**	**13.78**	**14.08**	**9.91**
3.27	**2.96**	**3.17**	**3.70**	**4.02**	**4.87**	**4.98**	**3.51**

Trial A	Trial A	Trial A	Trial A	Trial A	Trial A	Trial A	Trial A
HR 1st Int TM 2	HR 2nd Int TM 2	HR 3rd Int TM 2	HR Last 5 Min TM 2	HR 1st Int TM 3	HR 2nd Int TM 3	HR 3rd Int TM 3	HR Last 5 Min TM 3
148	143	136	121	152	147	140	121
158	158	147	131	156	159	155	136
XXXXX	XXXXX	XXXXX	XXXXX	XXXXX	XXXXX	XXXXX	XXXXX
127	125	148	132	148	142	141	125
157	151	144	130	158	153	147	130
159	155	151	139	159	158	155	139
134	133	126	110	133	131	123	111
158	159	152	132	158	157	155	136
148.71	**146.29**	**143.43**	**127.86**	**152.00**	**149.57**	**145.14**	**128.29**
13.14	**13.15**	**9.34**	**9.48**	**9.26**	**10.29**	**11.75**	**10.00**
4.64	**4.65**	**3.30**	**3.35**	**3.27**	**3.64**	**4.16**	**3.53**
Trial B	Trial B	Trial B	Trial B	Trial B	Trial B	Trial B	Trial B
HR 1st Int TM 2	HR 2nd Int TM 2	HR 3rd Int TM 2	HR Last 5 Min TM 2	HR 1st Int TM 3	HR 2nd Int TM 3	HR 3rd Int TM 3	HR Last 5 Min TM 3
135	133	126	111	138	132	128	114
156	154	154	132	161	160	156	138
159	153	148	128	160	151	149	135
148	147	142	126	146	146	140	124
163	161	157	144	169	164	162	145
146	143	137	123	143	142	140	126
138	132	128	116	139	137	127	115
142	136	132	122	146	148	142	129
148.38	**144.88**	**140.50**	**125.25**	**150.25**	**147.50**	**143.00**	**128.25**
10.13	**10.71**	**11.74**	**10.07**	**11.51**	**10.85**	**12.34**	**10.85**
3.58	**3.79**	**4.15**	**3.56**	**4.07**	**3.84**	**4.36**	**3.83**

Trial A	Trial A	Trial A	Trial A	Trial A	Trial A	Trial A	Trial A
HR 1st Int TM 4	HR 2nd Int TM 4	HR 3rd Int TM 4	HR Last 5 Min TM 4	HR 1st Int TM 5	HR 2nd Int TM 5	HR 3rd Int TM 5	HR Last 5 Min TM 5
154	148	141	124	155	155	149	128
161	161	155	139	162	164	162	143
XXXXX	XXXXX	XXXXX	XXXXX	XXXXX	XXXXX	XXXXX	158
152	157	129	124	154	156	151	124
159	153	151	131	159	153	151	139
162	159	154	142	161	161	158	150
135	131	125	115	134	133	128	115
158	156	151	134	157	158	157	136
154.43	**152.14**	**143.71**	**129.86**	**154.57**	**154.29**	**150.86**	**136.63**
9.29	**10.24**	**12.34**	**9.48**	**9.54**	**10.09**	**11.10**	**14.06**
3.28	**3.62**	**4.36**	**3.35**	**3.37**	**3.57**	**3.92**	**4.97**
Trial B	Trial B	Trial B	Trial B	Trial B	Trial B	Trial B	Trial B
HR 1st Int TM 4	HR 2nd Int TM 4	HR 3rd Int TM 4	HR Last 5 Min TM 4	HR 1st Int TM 5	HR 2nd Int TM 5	HR 3rd Int TM 5	HR Last 5 Min TM 5
138	138	132	119	145	141	139	126
163	166	160	147	165	167	161	154
165	161	152	139	167	166	161	144
146	149	144	130	150	149	142	130
175	170	165	150	180	176	171	158
153	150	146	137	158	145	150	143
140	134	128	118	144	142	136	123
149	136	146	122	156	158	135	135
153.63	**150.50**	**146.63**	**132.75**	**158.13**	**155.50**	**149.38**	**139.13**
13.00	**14.00**	**12.61**	**12.46**	**12.23**	**13.17**	**13.55**	**12.81**
4.60	**4.95**	**4.46**	**4.41**	**4.32**	**4.66**	**4.79**	**4.53**

Trial A	Trial A	Trial A	Trial A	Trial A	Trial A	Trial A	Trial A
HR 1st Int TM 6	HR 2nd Int TM 6	HR 3rd Int TM 6	HR Last 5 Min TM 6	HR 1st Int TM 7	HR 2nd Int TM 7	HR 3rd Int TM 7	HR Last 5 Min TM 7
155	151	146	127	148	142	139	128
157	161	159	141	160	161	162	146
XXXXX	XXXXX	XXXXX	XXXXX	XXXXX	XXXXX	XXXXX	XXXXX
149	144	127	127	161	156	148	126
160	157	153	140	162	157	151	140
160	158	158	151	151	158	154	150
137	131	125	112	133	130	125	110
153	152	149	132	153	153	153	133
153.00	**150.57**	**145.29**	**132.86**	**152.57**	**151.00**	**147.43**	**133.29**
8.06	**10.28**	**13.96**	**12.59**	**10.18**	**11.08**	**12.07**	**13.60**
2.85	**3.63**	**4.94**	**4.45**	**3.60**	**3.92**	**4.27**	**4.81**
Trial B	Trial B	Trial B	Trial B	Trial B	Trial B	Trial B	Trial B
HR 1st Int TM 6	HR 2nd Int TM 6	HR 3rd Int TM 6	HR Last 5 Min TM 6	HR 1st Int TM 7	HR 2nd Int TM 7	HR 3rd Int TM 7	HR Last 5 Min TM 7
145	142	137	123	148	144	140	127
165	159	162	151	159	157	156	128
165	158	162	151	168	166	162	152
144	145	140	125	142	145	140	123
174	172	168	153	174	169	163	151
155	152	146	140	155	152	150	143
135	134	128	118	136	129	128	114
158	150	140	133	158	155	149	133
155.13	**151.50**	**147.88**	**136.75**	**155.00**	**152.13**	**148.50**	**133.88**
13.04	**11.71**	**14.37**	**14.01**	**12.73**	**12.88**	**12.05**	**13.63**
4.61	**4.14**	**5.08**	**4.95**	**4.50**	**4.55**	**4.26**	**4.82**

Trial A	Trial A	Trial A	Trial A	Trial A	Trial A	Trial A	Trial A
HR 1st Int TM 8	HR 2nd Int TM 8	HR 3rd Int TM 8	HR Last 5 Min TM 8	RPE 1st Int Cyc 1	RPE 2nd Int Cyc 1	RPE 3rd Int Cyc 1	RPE Last 5 Min Cyc 1
149	148	142	136	13	12	11	10
149	144	138	123	13	13	12	11
XXXXX	XXXXX	XXXXX	166	13	12	12	9
157	157	144	132	14	14	14	11
163	162	160	140	13	14	14	11
153	154	151	148	11	12	13	11
137	134	128	114	13	12	11	10
152	151	146	134	13	13	13	11
151.43	**150.00**	**144.14**	**136.63**	**12.88**	**12.75**	**12.50**	**10.50**
8.04	**9.18**	**10.04**	**15.72**	**0.83**	**0.89**	**1.20**	**0.76**
2.84	**3.25**	**3.55**	**5.56**	**0.30**	**0.31**	**0.42**	**0.27**
Trial B	Trial B	Trial B	Trial B	Trial B	Trial B	Trial B	Trial B
HR 1st Int TM 8	HR 2nd Int TM 8	HR 3rd Int TM 8	HR Last 5 Min TM 8	RPE 1st Int Cyc 1	RPE 2nd Int Cyc 1	RPE 3rd Int Cyc 1	RPE Last 5 Min Cyc 1
147	145	143	132	12	12	12	12
159	163	159	144	13	12	12	12
164	163	139	XXXXX	12	12	11	11
145	143	141	127	14	14	13	11
165	161	160	155	14	15	13	12
151	150	148	127	12	12	11	10
142	135	137	121	13	12	12	9
164	163	164	141	14	14	12	11
154.63	**152.88**	**148.88**	**135.29**	**13.00**	**12.88**	**12.00**	**11.00**
9.46	**11.09**	**10.63**	**11.90**	**0.93**	**1.25**	**0.76**	**1.07**
3.34	**3.92**	**3.76**	**4.21**	**0.33**	**0.44**	**0.27**	**0.38**

Trial A	Trial A	Trial A	Trial A	Trial A	Trial A	Trial A	Trial A
RPE 1st Int Cyc 4	RPE 2nd Int Cyc 4	RPE 3rd Int Cyc 4	RPE Last 5 Min Cyc 4	RPE 1st Int Cyc 5	RPE 2nd Int Cyc 5	RPE 3rd Int Cyc 5	RPE Last 5 Min Cyc 5
13	12	11	12	14	12	11	10
16	15	14	13	15	14	14	13
14	13	13	11	14	13	12	11
15	15	13	11	14	14	13	11
13	13	13	11	15	14	13	12
13	13	12	11	14	13	13	11
14	13	12	10	14	13	12	11
13	12	11	11	13	13	12	11
13.88	**13.25**	**12.38**	**11.25**	**14.13**	**13.25**	**12.50**	**11.25**
1.13	**1.16**	**1.06**	**0.89**	**0.64**	**0.71**	**0.93**	**0.89**
0.40	**0.41**	**0.38**	**0.31**	**0.23**	**0.25**	**0.33**	**0.31**
Trial B	Trial B	Trial B	Trial B	Trial B	Trial B	Trial B	Trial B
RPE 1st Int Cyc 4	RPE 2nd Int Cyc 4	RPE 3rd Int Cyc 4	RPE Last 5 Min Cyc 4	RPE 1st Int Cyc 5	RPE 2nd Int Cyc 5	RPE 3rd Int Cyc 5	RPE Last 5 Min Cyc 5
14	14	14	12	13	14	13	12
15	15	14	13	16	15	14	13
15	14	14	12	17	16	14	12
15	15	14	11	15	15	12	11
14	14	13	12	14	15	16	12
13	12	12	11	14	13	12	11
15	14	13	10	15	13	11	10
13	12	12	11	13	12	11	11
14.25	**13.75**	**13.25**	**11.50**	**14.63**	**14.13**	**12.88**	**11.50**
0.89	**1.16**	**0.89**	**0.93**	**1.41**	**1.36**	**1.73**	**0.93**
0.31	**0.41**	**0.31**	**0.33**	**0.50**	**0.48**	**0.61**	**0.33**

Trial A	Trial A	Trial A	Trial A	Trial A	Trial A	Trial A	Trial A
RPE 1st Int Cyc 8	RPE 2nd Int Cyc 8	RPE 3rd Int Cyc 8	RPE Last 5 Min Cyc 8	RPE 1st Int TM 1	RPE 2nd Int TM 1	RPE 3rd Int TM 1	RPE Last 5 Min TM 1
15	14	13	12	12	11	10	9
19	18	18	18	13	13	12	11
17	16	16	13	13	13	12	10
18	18	17	14	14	15	14	11
16	16	15	12	14	13	14	11
14	14	14	13	13	12	12	11
14	13	12	11	13	12	12	11
14	12	12	11	12	12	12	11
15.88	**15.13**	**14.63**	**13.00**	**13.00**	**12.63**	**12.25**	**10.63**
1.96	**2.23**	**2.26**	**2.27**	**0.76**	**1.19**	**1.28**	**0.74**
0.69	**0.79**	**0.80**	**0.80**	**0.27**	**0.42**	**0.45**	**0.26**
Trial B	Trial B	Trial B	Trial B	Trial B	Trial B	Trial B	Trial B
RPE 1st Int Cyc 8	RPE 2nd Int Cyc 8	RPE 3rd Int Cyc 8	RPE Last 5 Min Cyc 8	RPE 1st Int TM 1	RPE 2nd Int TM 1	RPE 3rd Int TM 1	RPE Last 5 Min TM 1
17	15	14	14	13	13	13	12
19	18	18	17	13	13	12	12
17	16	15	18	13	13	13	11
17	18	19	15	15	14	13	11
18	18	19	18	15	15	15	11
14	13	13	12	13	12	12	11
13	13	12	12	13	14	13	11
13	13	13	12	13	13	13	11
16.00	**15.50**	**15.38**	**14.75**	**13.50**	**13.38**	**13.00**	**11.25**
2.33	**2.33**	**2.88**	**2.66**	**0.93**	**0.92**	**0.93**	**0.46**
0.82	**0.82**	**1.02**	**0.94**	**0.33**	**0.32**	**0.33**	**0.16**

Trial A	Trial A	Trial A	Trial A	Trial A	Trial A	Trial A	Trial A
RPE 1st Int TM 4	RPE 2nd Int TM 4	RPE 3rd Int TM 4	RPE Last 5 Min TM 4	RPE 1st Int TM 5	RPE 2nd Int TM 5	RPE 3rd Int TM 5	RPE Last 5 Min TM 5
13	12	11	10	13	13	12	11
15	14	13	12	15	15	14	13
14	14	12	11	15	14	14	12
15	14	13	11	14	14	13	11
15	13	13	12	15	15	14	12
13	12	12	12	14	14	13	13
14	13	12	11	14	13	12	11
12	12	12	11	12	12	11	10
13.88	**13.00**	**12.25**	**11.25**	**14.00**	**13.75**	**12.88**	**11.63**
1.13	**0.93**	**0.71**	**0.71**	**1.07**	**1.04**	**1.13**	**1.06**
0.40	**0.33**	**0.25**	**0.25**	**0.38**	**0.37**	**0.40**	**0.38**
Trial B	Trial B	Trial B	Trial B	Trial B	Trial B	Trial B	Trial B
RPE 1st Int TM 4	RPE 2nd Int TM 4	RPE 3rd Int TM 4	RPE Last 5 Min TM 4	RPE 1st Int TM 5	RPE 2nd Int TM 5	RPE 3rd Int TM 5	RPE Last 5 Min TM 5
14	14	14	12	14	12	14	12
15	13	13	11	15	14	14	13
16	15	15	13	17	15	14	13
16	15	14	11	16	15	14	11
15	15	14	11	16	17	14	12
13	12	12	11	14	13	13	12
14	13	12	10	15	13	12	11
11	11	11	10	12	11	11	11
14.25	**13.50**	**13.13**	**11.13**	**14.88**	**13.75**	**13.25**	**11.88**
1.67	**1.51**	**1.36**	**0.99**	**1.55**	**1.91**	**1.16**	**0.83**
0.59	**0.53**	**0.48**	**0.35**	**0.55**	**0.67**	**0.41**	**0.30**

Trial A	Trial A	Trial A	Trial A	Trial A	Trial A	Trial A	Trial A
RPE 1st Int TM 8	RPE 2nd Int TM 8	RPE 3rd Int TM 8	RPE Last 5 Min TM 8	VO2 (L/min) Cyc 1	VO2 (L/min) Cyc 4	VO2 (L/min) Cyc 5	VO2 (L/min) Cyc 8
12	12	12	11	2.366089	2.306114	2.08737	2.507431
18	18	17	17	2.358239	2.383088	2.345097	1.741045
18	18	18	16	2.176827	2.291836	2.267717	2.377555
17	16	15	11	2.070538	2.048493	2.047048	2.177187
15	14	13	11	2.377406	2.317701	2.439941	2.764799
15	14	14	14	2.012825	2.013397	2.039834	2.197619
14	13	12	11	2.314262	XXXXXX	2.310996	3.14731
11	11	11	9	2.072051	2.000757	1.976194	2.010339
15.00	**14.50**	**14.00**	**12.50**	**2.22**	**2.19**	**2.19**	**2.37**
2.62	**2.62**	**2.51**	**2.83**	**0.15**	**0.17**	**0.17**	**0.44**
0.93	**0.93**	**0.89**	**1.00**	**0.05**	**0.06**	**0.06**	**0.16**
Trial B	Trial B	Trial B	Trial B	Trial B	Trial B	Trial B	Trial B
RPE 1st Int TM 8	RPE 2nd Int TM 8	RPE 3rd Int TM 8	RPE Last 5 Min TM 8	VO2 (L/min) Cyc 1	VO2 (L/min) Cyc 4	VO2 (L/min) Cyc 5	VO2 (L/min) Cyc 8
15	14	13	12	2.339209	2.281713	2.320451	2.507786
17	17	17	16	2.370697	2.385082	2.422456	2.526468
19	18	XXXXXX	XXXXXX	2.203896	2.342254	2.320783	2.090583
18	17	17	14	1.943444	1.935095	1.986034	2.076291
20	20	20	19	2.318248	2.311284	2.396642	2.542278
14	14	14	13	2.06649	2.083497	2.093039	2.179132
14	13	12	11	2.333066	2.266795	2.307571	2.775375
12	12	12	11	2.179523	2.226378	2.156403	2.301827
16.13	**15.63**	**15.00**	**13.71**	**2.22**	**2.23**	**2.25**	**2.37**
2.80	**2.77**	**3.06**	**2.93**	**0.15**	**0.15**	**0.15**	**0.25**
0.99	**0.98**	**1.08**	**1.04**	**0.05**	**0.05**	**0.05**	**0.09**

Trial A	Trial A	Trial A	Trial A	Trial A	Trial A	Trial A	Trial A
VCO2 (L/min) Cyc 1	VCO2 (L/min) Cyc 4	VCO2 (L/min) Cyc 5	VCO2 (L/min) Cyc 8	RER Cyc 1	RER Cyc 4	RER Cyc 5	RER Cyc 8
2.139098	2.081945	1.871652	2.277737	0.904065	0.902794	0.896656	0.908395
2.072017	2.102749	2.091136	1.515717	0.878629	0.882363	0.891706	0.870579
2.036427	2.063226	2.059365	2.051193	0.935502	0.90025	0.908123	0.862732
1.800479	1.816600	1.868298	1.851422	0.869571	0.886798	0.912679	0.850373
2.108129	2.105845	2.230489	2.41013	0.886735	0.908592	0.914157	0.871720
1.802998	1.801439	1.881481	1.856801	0.895755	0.894726	0.922370	0.844915
2.047781	XXXXXX	2.132408	2.995428	0.884853	XXXXXX	0.922722	0.951742
1.849544	1.815811	1.859802	1.881569	0.892615	0.907562	0.941103	0.935946
1.98	**1.97**	**2.00**	**2.10**	**0.89**	**0.90**	**0.91**	**0.89**
0.14	**0.15**	**0.15**	**0.45**	**0.02**	**0.01**	**0.02**	**0.04**
0.05	**0.05**	**0.05**	**0.16**	**0.01**	**0.00**	**0.01**	**0.01**
Trial B	Trial B	Trial B	Trial B	Trial B	Trial B	Trial B	Trial B
VCO2 (L/min) Cyc 1	VCO2 (L/min) Cyc 4	VCO2 (L/min) Cyc 5	VCO2 (L/min) Cyc 8	RER Cyc 1	RER Cyc 4	RER Cyc 5	RER Cyc 8
2.064453	1.935624	1.947975	1.963704	0.882543	0.848321	0.839481	0.783043
2.144013	2.07206	2.088334	2.142725	0.904381	0.868758	0.862073	0.848111
2.039167	2.02104	2.048104	1.665586	0.925256	0.862861	0.882506	0.796709
1.817353	1.688889	1.771106	1.719498	0.935120	0.872768	0.89178	0.828158
2.056969	2.001379	2.119042	2.08335	0.887295	0.865917	0.884171	0.819482
1.815502	1.742928	1.825822	1.72457	0.878544	0.83654	0.872331	0.791402
2.003592	1.876972	2.01372	2.327589	0.858781	0.828029	0.872658	0.838657
1.949259	1.937585	1.911119	1.916837	0.894351	0.870286	0.886253	0.832746
1.99	**1.91**	**1.97**	**1.94**	**0.90**	**0.86**	**0.87**	**0.82**
0.12	**0.13**	**0.12**	**0.23**	**0.03**	**0.02**	**0.02**	**0.02**
0.04	**0.05**	**0.04**	**0.08**	**0.01**	**0.01**	**0.01**	**0.01**

Trial A	Trial A	Trial A	Trial A	Trial A	Trial A	Trial A
CHO g/min Cyc 1	CHO g/min Cyc 4	CHO g/min Cyc 5	CHO g/min Cyc 8	FAT g/min Cyc 1	FAT g/min Cyc 4	FAT g/min Cyc 5
2.137750	2.070224	1.815559	2.314850	0.379075	0.374362	0.360249
1.857730	1.917795	1.986907	1.307758	0.477991	0.468166	0.424115
2.278128	2.030885	2.090739	1.700977	0.234468	0.381779	0.347948
1.545752	1.689867	1.929732	1.435200	0.450999	0.387261	0.298513
1.960514	2.141775	2.316514	2.091087	0.449693	0.353800	0.349785
1.742473	1.733543	2.012871	1.394088	0.350411	0.353970	0.264450
1.888623	2.086391	2.284159	3.526332	0.445023	XXXXXX	0.298242
1.764141	1.839510	2.118516	2.107951	0.371587	0.308860	0.194375
1.90	**1.94**	**2.07**	**1.98**	**0.39**	**0.38**	**0.32**
0.23	**0.17**	**0.17**	**0.73**	**0.08**	**0.05**	**0.07**
0.08	**0.06**	**0.06**	**0.26**	**0.03**	**0.02**	**0.02**
Trial B	Trial B	Trial B	Trial B	Trial B	Trial B	Trial B
CHO g/min Cyc 1	CHO g/min Cyc 4	CHO g/min Cyc 5	CHO g/min Cyc 8	FAT g/min Cyc 1	FAT g/min Cyc 4	FAT g/min Cyc 5
1.884400	1.482790	1.414639	0.884860	0.458843	0.577969	0.622035
2.145322	1.771760	1.725836	1.639436	0.378562	0.522747	0.557984
2.203704	1.677097	1.869160	0.867645	0.275097	0.536427	0.455374
2.030501	1.472790	1.683363	1.158822	0.210572	0.411164	0.358930
1.917633	1.687053	1.948420	1.318530	0.436336	0.517541	0.463592
1.627101	1.242297	1.588835	0.851780	0.419150	0.568750	0.446252
1.627202	1.263811	1.755123	1.681576	0.550222	0.651004	0.490731
1.872860	1.669338	1.773538	1.332744	0.384541	0.482284	0.409624
1.91	**1.53**	**1.72**	**1.22**	**0.39**	**0.53**	**0.48**
0.21	**0.20**	**0.16**	**0.34**	**0.11**	**0.07**	**0.08**
0.08	**0.07**	**0.06**	**0.12**	**0.04**	**0.02**	**0.03**

Trial A	Trial A	Trial A	Trial A	Trial A	Trial A	Trial A	Trial A
FAT g/min Cyc 8	CHO kcal/min Cyc 1	CHO kcal/min Cyc 4	CHO kcal/min Cyc 5	CHO kcal/min Cyc 8	FAT kcal/min Cyc 1	FAT kcal/min Cyc 4	FAT kcal/min Cyc 5
0.383589	8.551001	8.280895	7.262236	9.259399	3.411675	3.369260	3.242242
0.376298	7.430921	7.671182	7.947630	5.231032	4.301917	4.213495	3.817034
0.545025	9.112513	8.123539	8.362957	6.803906	2.110212	3.436008	3.131531
0.544028	6.183010	6.759470	7.718927	5.740799	4.058987	3.485352	2.686613
0.592297	7.842055	8.567098	9.266057	8.364347	4.047233	3.184196	3.148064
0.569166	6.969891	6.934172	8.051486	5.576350	3.153700	3.185729	2.380046
0.253643	7.554490	XXXXXX	9.136637	14.105329	4.005209	XXXXXX	2.684178
0.215046	7.056566	7.358040	8.474065	8.431803	3.344280	2.779738	1.749372
0.43	**7.59**	**7.67**	**8.28**	**7.94**	**3.55**	**3.38**	**2.85**
0.15	**0.92**	**0.69**	**0.68**	**2.91**	**0.71**	**0.44**	**0.63**
0.05	**0.33**	**0.24**	**0.24**	**1.03**	**0.25**	**0.15**	**0.22**
Trial B	Trial B	Trial B	Trial B	Trial B	Trial B	Trial B	Trial B
FAT g/min Cyc 8	CHO kcal/min Cyc 1	CHO kcal/min Cyc 4	CHO kcal/min Cyc 5	CHO kcal/min Cyc 8	FAT kcal/min Cyc 1	FAT kcal/min Cyc 4	FAT kcal/min Cyc 5
0.908617	7.537601	5.931162	5.658554	3.539441	4.129583	5.201718	5.598314
0.640851	8.581287	7.087039	6.903344	6.557746	3.407061	4.704721	5.021854
0.709745	8.814815	6.708387	7.476639	3.470579	2.475877	4.827846	4.098365
0.595844	8.122004	5.891160	6.733453	4.635287	1.895148	3.700476	3.230368
0.766410	7.670531	6.748211	7.793681	5.274120	3.927023	4.657872	4.172328
0.759119	6.508405	4.969188	6.355340	3.407119	3.772350	5.118752	4.016272
0.747803	6.508807	5.055243	7.020492	6.726305	4.951994	5.859040	4.416581
0.642933	7.491438	6.677353	7.094151	5.330975	3.460868	4.340559	3.686619
0.72	**7.65**	**6.13**	**6.88**	**4.87**	**3.50**	**4.80**	**4.28**
0.10	**0.85**	**0.80**	**0.66**	**1.34**	**0.96**	**0.64**	**0.74**
0.03	**0.30**	**0.28**	**0.23**	**0.47**	**0.34**	**0.22**	**0.26**

Trial A	Trial A	Trial A	Trial A	Trial A	Trial A	Trial A	Trial A
FAT kcal/min Cyc 8	Total kcal/min Cyc 1	Total kcal/min Cyc 4	Total kcal/min Cyc 5	Total kcal/min Cyc 8	% CHO Cyc 1	% CHO Cyc 4	% CHO Cyc 5
3.452301	11.962676	11.650155	10.504477	12.711700	71.480672	71.079698	69.134670
3.386680	11.732837	11.884677	11.764664	8.617711	63.334387	64.546827	67.555096
4.905221	11.222725	11.559547	11.494487	11.709127	81.196973	70.275581	72.756240
4.896248	10.241997	10.244822	10.405540	10.637047	60.369185	65.979381	74.180941
5.330675	11.889288	11.751294	12.414121	13.695022	65.958994	72.903446	74.641269
5.122495	10.123590	10.119901	10.431531	10.698845	68.848011	68.520159	77.184121
2.282786	11.559700	XXXXXX	11.820815	16.388116	65.351959	XXXXXX	77.292786
1.935413	10.400846	10.137779	10.223437	10.367216	67.846076	72.580400	82.888614
3.91	**11.14**	**11.05**	**11.13**	**11.85**	**68.05**	**69.41**	**74.45**
1.34	**0.77**	**0.83**	**0.84**	**2.40**	**6.30**	**3.21**	**4.86**
0.47	**0.27**	**0.29**	**0.30**	**0.85**	**2.23**	**1.14**	**1.72**
Trial B	Trial B	Trial B	Trial B	Trial B	Trial B	Trial B	Trial B
FAT kcal/min Cyc 8	Total kcal/min Cyc 1	Total kcal/min Cyc 4	Total kcal/min Cyc 5	Total kcal/min Cyc 8	% CHO Cyc 1	% CHO Cyc 4	% CHO Cyc 5
8.177552	11.667184	11.132880	11.256868	11.716993	64.605146	53.276081	50.267569
5.767657	11.988348	11.791760	11.925197	12.325403	71.580233	60.101624	57.888717
6.387705	11.290692	11.536233	11.575004	9.858284	78.071522	58.150582	64.592969
5.362599	10.017151	9.591636	9.963820	9.997886	81.080971	61.419761	67.579024
6.897688	11.597555	11.406083	11.966009	12.171808	66.139213	59.163264	65.131833
6.832067	10.280754	10.087940	10.371611	10.239186	63.306685	49.258699	61.276301
6.730224	11.460801	10.914282	11.437073	13.456528	56.791902	46.317682	61.383646
5.786400	10.952306	11.017912	10.780770	11.117374	68.400556	60.604526	65.803754
6.49	**11.16**	**10.93**	**11.16**	**11.36**	**68.75**	**56.04**	**61.74**
0.89	**0.69**	**0.75**	**0.73**	**1.28**	**7.96**	**5.72**	**5.57**
0.31	**0.25**	**0.26**	**0.26**	**0.45**	**2.81**	**2.02**	**1.97**

Trial A	Trial A	Trial A	Trial A	Trial A	Trial A	Trial A	Trial A
% CHO Cyc 8	% FAT Cyc 1	% FAT Cyc 4	% FAT Cyc 5	% FAT Cyc 8	VO2 (L/min) TM 1	VO2 (L/min) TM 4	VO2 (L/min) TM 5
72.841549	28.519328	28.920302	30.865330	27.158451	2.143386	2.148255	2.151067
60.700937	36.665613	35.453173	32.444904	39.29906	1.983679	2.007776	2.056658
58.107716	18.803027	29.724419	27.243760	41.892284	2.481744	2.548488	2.631060
53.969858	39.630815	34.020619	25.819059	46.030142	1.951679	1.893507	1.991684
61.075819	34.041006	27.096554	25.358731	38.924181	2.451394	2.437685	2.424886
52.121050	31.151989	31.479841	22.815879	47.87895	1.961998	1.972899	2.091913
86.070476	34.648041	XXXXXX	22.707214	13.929524	2.397945	2.270861	2.304420
81.331410	32.153924	27.419600	17.111386	18.668590	1.806068	1.788642	1.811211
65.78	**31.95**	**30.59**	**25.55**	**34.22**	**2.15**	**2.13**	**2.18**
12.74	**6.30**	**3.21**	**4.86**	**12.74**	**0.26**	**0.27**	**0.26**
4.50	**2.23**	**1.14**	**1.72**	**4.50**	**0.09**	**0.09**	**0.09**
Trial B	Trial B	Trial B	Trial B	Trial B	Trial B	Trial B	Trial B
% CHO Cyc 8	% FAT Cyc 1	% FAT Cyc 4	% FAT Cyc 5	% FAT Cyc 8	VO2 (L/min) TM 1	VO2 (L/min) TM 4	VO2 (L/min) TM 5
30.207755	35.394854	46.723919	49.732431	69.79224	2.136038	2.241521	2.223027
53.205123	28.419767	39.898376	42.111283	46.79488	1.959808	2.002237	2.080645
35.204700	21.928478	41.849418	35.407031	64.79530	2.367784	2.401815	2.487655
46.362673	18.919029	38.580239	32.420976	53.63733	1.974364	1.950173	1.971661
43.330624	33.860787	40.836736	34.868167	56.66938	2.627770	2.533887	2.644822
33.275293	36.693315	50.741301	38.723699	66.72471	1.991565	2.003931	2.095295
49.985439	43.208098	53.682318	38.616354	50.01456	2.442497	2.478612	2.462017
47.951742	31.599444	39.395474	34.196246	52.04826	1.787257	1.862563	1.871078
42.44	**31.25**	**43.96**	**38.26**	**57.56**	**2.16**	**2.18**	**2.23**
8.50	**7.96**	**5.72**	**5.57**	**8.50**	**0.29**	**0.26**	**0.27**
3.00	**2.81**	**2.02**	**1.97**	**3.00**	**0.10**	**0.09**	**0.10**

Trial A	Trial A	Trial A	Trial A	Trial A	Trial A	Trial A	Trial A
VO2 (L/min) TM 8	VCO2 (L/min) TM 1	VCO2 (L/min) TM 4	VCO2 (L/min) TM 5	VCO2 (L/min) TM 8	RER TM 1	RER TM 4	RER TM 5
2.344155	1.960472	1.940766	1.935494	2.013461	0.914661	0.903415	0.899783
1.512281	1.768418	1.784535	1.816702	1.324222	0.891484	0.888812	0.883327
2.915075	2.326912	2.284646	2.378702	2.567817	0.937612	0.896471	0.904085
1.930735	1.714796	1.684119	1.784709	1.630810	0.878626	0.889418	0.896080
2.541218	2.194387	2.239462	2.245879	2.228937	0.895159	0.918684	0.926179
2.200944	1.749316	1.768425	1.840141	1.848309	0.891599	0.896359	0.879645
2.300543	2.108554	2.027683	2.109648	2.086819	0.879317	0.892914	0.915479
1.824138	1.614018	1.649762	1.688388	1.686047	0.893664	0.922355	0.932187
2.20	**1.93**	**1.92**	**1.97**	**1.92**	**0.90**	**0.90**	**0.90**
0.44	**0.26**	**0.24**	**0.24**	**0.39**	**0.02**	**0.01**	**0.02**
0.15	**0.09**	**0.09**	**0.09**	**0.14**	**0.01**	**0.00**	**0.01**
Trial B	Trial B	Trial B	Trial B	Trial B	Trial B	Trial B	Trial B
VO2 (L/min) TM 8	VCO2 (L/min) TM 1	VCO2 (L/min) TM 4	VCO2 (L/min) TM 5	VCO2 (L/min) TM 8	RER TM 1	RER TM 4	RER TM 5
2.399052	1.85253	1.824078	1.802805	1.861452	0.867274	0.813768	0.810969
1.818668	1.754209	1.683739	1.697054	1.434578	0.895092	0.840929	0.815638
XXXXXX	2.162159	2.059569	2.187138	XXXXXX	0.913157	0.857505	0.879197
1.990186	1.810673	1.668107	1.747226	1.572371	0.917092	0.855364	0.886170
2.607763	2.314896	2.215600	2.237038	2.116581	0.880936	0.874388	0.845818
1.717603	1.772444	1.656798	1.784455	1.359973	0.889975	0.826774	0.851649
2.451079	2.136615	2.079362	2.149026	2.014897	0.874767	0.838922	0.872872
1.977393	1.591525	1.594843	1.618472	1.581215	0.890485	0.856263	0.864994
2.14	**1.92**	**1.85**	**1.90**	**1.71**	**0.89**	**0.85**	**0.85**
0.34	**0.25**	**0.24**	**0.25**	**0.29**	**0.02**	**0.02**	**0.03**
0.12	**0.09**	**0.08**	**0.09**	**0.10**	**0.01**	**0.01**	**0.01**

Trial A	Trial A	Trial A	Trial A	Trial A	Trial A	Trial A	Trial A
RER TM 8	CHO g/min TM 1	CHO g/min TM 4	CHO g/min TM 5	CHO g/min TM 8	FAT g/min TM 1	FAT g/min TM 4	FAT g/min TM 5
0.858928	2.039879	1.934587	1.901573	1.636510	0.305466	0.346507	0.360007
0.875645	1.678692	1.674673	1.664122	1.170788	0.359486	0.372812	0.400727
0.880875	2.621051	2.214493	2.377392	2.326177	0.258569	0.440616	0.421438
0.844658	1.537432	1.584584	1.727120	1.222526	0.395595	0.349678	0.345648
0.877114	2.115486	2.364583	2.434865	1.984354	0.429202	0.331032	0.298942
0.839780	1.661374	1.713328	1.657601	1.344776	0.355179	0.341472	0.420459
0.907098	1.896517	1.936494	2.201710	2.110283	0.483283	0.406107	0.325269
0.924298	1.546304	1.764876	1.868178	1.816031	0.320724	0.231930	0.205114
0.88	**1.89**	**1.90**	**1.98**	**1.70**	**0.36**	**0.35**	**0.35**
0.03	**0.37**	**0.27**	**0.32**	**0.43**	**0.07**	**0.06**	**0.07**
0.01	**0.13**	**0.10**	**0.11**	**0.15**	**0.03**	**0.02**	**0.03**
Trial B	Trial B	Trial B	Trial B	Trial B	Trial B	Trial B	Trial B
RER TM 8	CHO g/min TM 1	CHO g/min TM 4	CHO g/min TM 5	CHO g/min TM 8	FAT g/min TM 1	FAT g/min TM 4	FAT g/min TM 5
0.775911	1.572330	1.104272	1.066846	0.768650	0.473458	0.697130	0.701771
0.788807	1.690667	1.233832	1.042725	0.689406	0.343350	0.531892	0.640597
XXXXXX	2.237237	1.661213	1.966105	XXXXXX	0.343394	0.571551	0.501863
0.790062	1.900854	1.329832	1.620846	0.765791	0.273364	0.471050	0.374806
0.811646	2.097635	1.947203	1.688644	1.259524	0.522500	0.531539	0.680999
0.791785	1.671697	1.105812	1.393373	0.674372	0.365932	0.579712	0.519103
0.822045	1.881183	1.504753	1.874994	1.299818	0.510823	0.666748	0.522695
0.799646	1.504344	1.277708	1.357887	0.847097	0.326872	0.447092	0.421852
0.80	**1.82**	**1.40**	**1.50**	**0.90**	**0.39**	**0.56**	**0.55**
0.02	**0.26**	**0.29**	**0.35**	**0.27**	**0.09**	**0.09**	**0.12**
0.01	**0.09**	**0.10**	**0.12**	**0.09**	**0.03**	**0.03**	**0.04**

Trial A	Trial A	Trial A	Trial A	Trial A	Trial A	Trial A	Trial A
FAT g/min TM 8	CHO kcal/min TM 1	CHO kcal/min TM 4	CHO kcal/min TM 5	CHO kcal/min TM 8	FAT kcal/min TM 1	FAT kcal/min TM 4	FAT kcal/min TM 5
0.552259	8.159514	7.738347	7.606291	6.546040	2.749197	3.118560	3.240062
0.314059	6.714769	6.698693	6.656488	4.683152	3.235373	3.355312	3.606539
0.579921	10.484205	8.857971	9.509566	9.304706	2.327125	3.965545	3.792941
0.500875	6.149729	6.338336	6.908481	4.890105	3.560351	3.147102	3.110834
0.521509	8.461944	9.458333	9.739462	7.937414	3.862815	2.979292	2.690475
0.588900	6.645497	6.853312	6.630403	5.379103	3.196610	3.073244	3.784133
0.356919	7.586069	7.745975	8.806841	8.441134	4.349547	3.654965	2.927423
0.230612	6.185214	7.059505	7.472712	7.264123	2.886512	2.087366	1.846030
0.46	**7.55**	**7.59**	**7.92**	**6.81**	**3.27**	**3.17**	**3.12**
0.14	**1.47**	**1.09**	**1.27**	**1.72**	**0.65**	**0.55**	**0.65**
0.05	**0.52**	**0.39**	**0.45**	**0.61**	**0.23**	**0.19**	**0.23**
Trial B	Trial B	Trial B	Trial B	Trial B	Trial B	Trial B	Trial B
FAT g/min TM 8	CHO kcal/min TM 1	CHO kcal/min TM 4	CHO kcal/min TM 5	CHO kcal/min TM 8	FAT kcal/min TM 1	FAT kcal/min TM 4	FAT kcal/min TM 5
0.897792	6.289318	4.417090	4.267384	3.074599	4.261125	6.274168	6.315937
0.641430	6.762669	4.935327	4.170901	2.757622	3.090153	4.787025	5.765373
XXXXXX	8.948947	6.644851	7.864421	XXXXXX	3.090544	5.143957	4.516771
0.697751	7.603415	5.319326	6.483386	3.063164	2.460276	4.239452	3.373258
0.820274	8.390540	7.788811	6.754577	5.038097	4.702496	4.783854	6.128994
0.597242	6.686786	4.423250	5.573493	2.697486	3.293389	5.217409	4.671925
0.728424	7.524732	6.019010	7.499975	5.199271	4.597406	6.000728	4.704255
0.661617	6.017375	5.110834	5.431549	3.388387	2.941852	4.023832	3.796668
0.72	**7.28**	**5.58**	**6.01**	**3.60**	**3.55**	**5.06**	**4.91**
0.11	**1.03**	**1.17**	**1.38**	**1.06**	**0.84**	**0.78**	**1.07**
0.04	**0.36**	**0.41**	**0.49**	**0.38**	**0.30**	**0.28**	**0.38**

Trial A	Trial A	Trial A	Trial A	Trial A	Trial A	Trial A	Trial A
FAT kcal/min TM 8	Total kcal/min TM 1	Total kcal/min TM 4	Total kcal/min TM 5	Total kcal/min TM 8	% CHO TM 1	% CHO TM 4	% CHO TM 5
4.970331	10.908712	10.856907	10.846353	11.516371	74.798147	71.275799	70.127634
2.826527	9.950142	10.054005	10.263026	7.509679	67.484154	66.627109	64.858916
5.219288	12.811330	12.823517	13.302507	14.523994	81.835415	69.075992	71.487023
4.507873	9.710080	9.485438	10.019315	9.397977	63.333450	66.821756	68.951629
4.693583	12.324760	12.437625	12.429937	12.630998	68.658089	76.046136	78.35488
5.300104	9.842107	9.926556	10.414536	10.679207	67.521077	69.040177	63.664891
3.212272	11.935616	11.400941	11.734264	11.653405	63.558254	67.941546	75.05235
2.075508	9.071726	9.146872	9.318742	9.339631	68.181231	77.179450	80.190141
4.10	**10.82**	**10.77**	**11.04**	**10.91**	**69.42**	**70.50**	**71.59**
1.22	**1.39**	**1.36**	**1.34**	**2.18**	**6.14**	**4.06**	**5.96**
0.43	**0.49**	**0.48**	**0.47**	**0.77**	**2.17**	**1.43**	**2.11**
Trial B	Trial B	Trial B	Trial B	Trial B	Trial B	Trial B	Trial B
FAT kcal/min TM 8	Total kcal/min TM 1	Total kcal/min TM 4	Total kcal/min TM 5	Total kcal/min TM 8	% CHO TM 1	% CHO TM 4	% CHO TM 5
8.080128	10.550443	10.691258	10.583321	11.154727	59.611884	41.314968	40.32179
5.772873	9.852822	9.722352	9.936274	8.530495	68.636874	50.762685	41.97651
XXXXXX	12.039491	11.788809	12.381192	XXXXXX	74.329947	56.365757	63.51910
6.279759	10.063691	9.558778	9.856644	9.342923	75.552947	55.648599	65.77681
7.382465	13.093037	12.572665	12.883571	12.420563	64.083991	61.950360	52.42783
5.375179	9.980175	9.640659	10.245418	8.072665	67.000692	45.881197	54.39986
6.555815	12.122138	12.019738	12.204230	11.755087	62.074294	50.076053	61.45390
5.954555	8.959227	9.134665	9.228217	9.342942	67.163998	55.949874	58.85805
6.49	**10.83**	**10.64**	**10.91**	**10.09**	**67.31**	**52.24**	**54.84**
0.95	**1.42**	**1.32**	**1.37**	**1.68**	**5.56**	**6.58**	**9.54**
0.34	**0.50**	**0.47**	**0.48**	**0.59**	**1.96**	**2.33**	**3.37**

Trial A	Trial A	Trial A	Trial A	Trial A	Trial A	Trial A	Trial A
% CHO TM 8	% FAT TM 1	% FAT TM 4	% FAT TM 5	% FAT TM 8	Avg RPM's Cyc 1	Avg RPM's Cyc2	Avg RPM's Cyc 3
56.841171	25.20185	28.72420	29.87237	43.15883	XXXXX	XXXXX	XXXXX
62.361551	32.51585	33.37289	35.14108	37.63845	86	86	86
64.06438	18.16458	30.92401	28.51298	35.93562	73	73	82
52.03359	36.66655	33.17824	31.04837	47.96641	66	68	65
62.84075	31.34191	23.95386	21.64512	37.15925	78	81	81
50.36987	32.47892	30.95982	36.33511	49.63013	83	86	89
72.434910	36.44175	32.05845	24.94765	27.56509	88	88	87
77.777412	31.81877	22.82055	19.80986	22.22259	91	92	89
62.34	**30.58**	**29.50**	**28.41**	**37.66**	**80.71**	**82.00**	**82.71**
9.44	**6.14**	**4.06**	**5.96**	**9.44**	**8.90**	**8.62**	**8.42**
3.34	**2.17**	**1.43**	**2.11**	**3.34**	**3.15**	**3.05**	**2.98**
Trial B	Trial B	Trial B	Trial B	Trial B	Trial B	Trial B	Trial B
% CHO TM 8	% FAT TM 1	% FAT TM 4	% FAT TM 5	% FAT TM 8	Avg RPM's Cyc 1	Avg RPM's Cyc2	Avg RPM's Cyc 3
27.56319	40.38812	58.68503	59.67821	72.43681	XXXXX	XXXXX	XXXXX
32.32664	31.36313	49.23732	58.02349	67.67336	96	99	100
XXXXXX	25.67005	43.63424	36.48090	XXXXXX	74	73	76
32.78593	24.44705	44.35140	34.22319	67.21407	68	70	71
40.56255	35.91601	38.04964	47.57217	59.43745	68	69	76
33.41506	32.99931	54.11880	45.60014	66.58494	88	91	92
44.22997	37.92571	49.92395	38.54610	55.77003	87	84	86
36.26681	32.83600	44.05013	41.14195	63.73319	98	99	103
35.31	**32.69**	**47.76**	**45.16**	**64.69**	**82.71**	**83.57**	**86.29**
5.58	**5.56**	**6.58**	**9.54**	**5.58**	**12.68**	**13.16**	**12.55**
1.97	**1.96**	**2.33**	**3.37**	**1.97**	**4.48**	**4.65**	**4.44**

Trial A	Trial A	Trial A	Trial A	Trial A	Trial A	Trial A	Trial A
Avg RPM's Cyc 4	Avg RPM's Cyc 5	Avg RPM's Cyc 6	Avg RPM's Cyc 7	Avg RPM's Cyc 8	pre weight (kg)	post weight (kg)	weight difference (kg)
XXXXX	XXXXX	XXXXX	XXXXX	XXXXX	84.6	82.7	-1.9
86	86	74	43	35	63.8	61.8	-2.0
XXXXX	80	XXXXX	72	69	81.5	78.6	-2.9
63	70	64	57	58	67.0	66.4	-0.6
83	84	87	90	93	89.2	86.4	-2.8
88	88	91	89	88	65.2	64.6	-0.6
87	89	86	87	102	84.3	82.4	-1.9
86	84	85	93	86	61.7	61.1	-0.6
82.17	**83.00**	**81.17**	**75.86**	**75.86**	**74.66**	**73.00**	**-1.66**
9.54	**6.45**	**10.15**	**19.33**	**23.32**	**11.24**	**10.52**	**0.96**
3.37	**2.28**	**3.59**	**6.83**	**8.24**	**3.97**	**3.72**	**0.34**
Trial B	Trial B	Trial B	Trial B	Trial B	Trial B	Trial B	Trial B
Avg RPM's Cyc 4	Avg RPM's Cyc 5	Avg RPM's Cyc 6	Avg RPM's Cyc 7	Avg RPM's Cyc 8	pre weight (kg)	post weight (kg)	weight difference (kg)
XXXXX	XXXXX	XXXXX	XXXXX	XXXXX	84.4	82.7	-1.7
100	XXXXX	87	46	31	62.9	61.9	-1.0
71	69	71	70	42	81.3	78.6	-2.7
70	65	64	57	47	67.9	66.2	-1.7
77	79	80	82	76	89.6	86.1	-3.5
92	91	91	92	90	65.6	64.3	-1.3
84	82	86	91	96	84.8	82.2	-2.6
100	95	95	98	101	62.5	60.2	-2.3
84.86	**80.17**	**82.00**	**76.57**	**69.00**	**74.88**	**72.78**	**-2.10**
12.81	**11.81**	**11.11**	**19.56**	**28.58**	**11.20**	**10.63**	**0.83**
4.53	**4.17**	**3.93**	**6.92**	**10.10**	**3.96**	**3.76**	**0.29**

Trial A	Trial A	Trial A	Trial A	Trial A	Trial A	Trial A	Trial A
Total Void (L)	Urinary Nitrogen (mg/dL)	Time pre biopsy to Bkfast (min)	Time Bkfst to Exer (min)	Time Exer to post biopsy (min)	Blood Glucose Pre (mmol/L)	Blood Glucose Lunch (mmol/L)	Blood Glucose Post (mmol/L)
0.675	732	22.53	33.93	11.25	2.98	4.52	4.64
0.250	810	15.05	35.63	10.62	3.71	5.24	5.45
1.925	709	25.12	29.82	11.63	3.05	4.27	5.52
0.925	976	31.32	25.22	14.45	3.72	4.62	5.13
1.700	463	26.37	34.53	13.17	2.95	3.43	4.37
0.900	362	28.52	32.37	12.72	3.85	5.06	5.54
1.100	731	22.23	29.45	11.45	4.28	4.73	5.93
1.300	411	25.88	35.12	12.62	3.38	4.16	4.68
1.10	**649.25**	**24.63**	**32.01**	**12.24**	**3.49**	**4.50**	**5.16**
0.54	**215.03**	**4.88**	**3.59**	**1.24**	**0.48**	**0.57**	**0.54**
0.19	**76.03**	**1.73**	**1.27**	**0.44**	**0.17**	**0.20**	**0.19**
Trial B	Trial B	Trial B	Trial B	Trial B	Trial B	Trial B	Trial B
Total Void (L)	Urinary Nitrogen (mg/dL)	Time pre biopsy to Bkfast (min)	Time Bkfst to Exer (min)	Time Exer to post biopsy (min)	Blood Glucose Pre (mmol/L)	Blood Glucose Lunch (mmol/L)	Blood Glucose Post (mmol/L)
0.525	993	26.72	35.12	12.62	2.94	3.39	2.13
0.300	1049	21.85	36.05	9.28	3.59	4.01	3.14
2.050	372	23.27	30.37	XXXXX	2.95	2.77	2.62
1.250	408	24.15	27.43	16.17	3.22	2.95	2.35
1.275	353	28.53	36.78	12.18	3.81	3.31	2.72
1.325	449	22.88	31.08	9.85	4.26	4.33	2.01
0.725	804	21.78	33.82	12.62	4.10	4.10	4.15
1.775	342	21.58	33.03	10.57	3.27	3.38	3.93
1.15	**596.25**	**23.85**	**32.96**	**11.90**	**3.52**	**3.53**	**2.88**
0.60	**301.61**	**2.53**	**3.17**	**2.32**	**0.51**	**0.56**	**0.80**
0.21	**106.63**	**0.90**	**1.12**	**0.82**	**0.18**	**0.20**	**0.28**

Adjusted for % recovery

Trial A	Trial A	Trial A	Trial A	Trial A	Trial A	Trial A	Trial A
Insulin Pre (µIU/mL)	Insulin Lunch (µIU/mL)	Insulin Post (µIU/mL)	Lactate Pre	Lactate Lunch	Lactate Post	Muscle Glycogen pre (mmol/g wet)	Muscle Glycogen post (mmol/g wet)
5.587	16.093	13.351	1.55	1.53	1.88	125.0099	51.44777
5.696	7.964	7.590	1.68	1.95	2.34	124.4962	49.40293
4.837	13.197	14.440	1.74	1.67	2.44	164.2432	77.06331
3.434	10.840	8.180	2.05	3.09	2.24	108.2394	67.21273
5.779	16.183	13.637	1.23	1.37	1.79	115.2567	21.50329
7.028	14.128	13.199	1.22	1.73	1.81	103.6237	22.28795
3.393	8.208	11.081	1.41	1.74	1.54	228.7633	163.0158
4.622	21.565	15.882	2.16	1.82	1.90	230.41	143.66
5.05	**13.52**	**12.17**	**1.63**	**1.86**	**1.99**	**150.00**	**74.45**
1.32	**3.45**	**2.77**	**0.35**	**0.53**	**0.31**	**52.44**	**52.61**
0.47	**1.22**	**0.98**	**0.12**	**0.19**	**0.11**	**18.54**	**18.60**
Trial B	Trial B	Trial B	Trial B	Trial B	Trial B	Trial B	Trial B
Insulin Pre (µIU/mL)	Insulin Lunch (µIU/mL)	Insulin Post (µIU/mL)	Lactate Pre (mmol/L)	Lactate Lunch (mmol/L)	Lactate Post (mmol/L)	Muscle Glycogen pre (mmol/g wet)	Muscle Glycogen post (mmol/g wet)
3.633	3.095	2.880	1.52	1.50	2.07	134.2353	30.09238
6.314	4.354	3.753	1.38	2.55	4.26	101.2918	31.75072
3.933	3.562	2.701	1.52	1.73	2.59	222.2708	73.41455
3.970	4.150	2.641	2.28	3.82	3.41	129.1247	34.26359
8.511	5.867	2.703	2.60	1.86	2.30	187.2168	17.51272
9.023	4.309	2.045	1.61	1.52	2.29	101.9649	16.52
3.684	4.476	2.442	1.38	1.62	1.57	217.38	143.85
5.950	5.754	2.992	1.87	1.84	2.31	189.95	57.27
5.63	**4.45**	**2.77**	**1.77**	**2.06**	**2.60**	**160.43**	**50.58**
2.20	**0.96**	**0.49**	**0.45**	**0.79**	**0.85**	**49.62**	**42.36**
0.78	**0.34**	**0.17**	**0.16**	**0.28**	**0.30**	**17.54**	**14.98**

Raw

Trial A	Trial A	Trial A	Trial A	Trial A
Muscle Glycogen pre (mmol/g wet)	Muscle Glycogen post (mmol/g wet)	Change (pre to post) (mmol/g wet)	% Change (pre to post)	Rate of Muscle Glycogenolysis
112.5089	46.3030	73.56	-143%	9.20
112.0465	44.4626	75.09	-152%	9.39
147.8189	69.3570	87.18	-113%	10.90
97.4154	60.4915	41.03	-61%	5.13
103.7310	19.3530	93.75	-436%	11.72
93.2613	20.0592	81.34	-365%	10.17
205.8869	146.7142	65.75	-40%	8.22
207.3650	129.2982	86.74	-60%	10.84
135.00	**67.00**	**75.55**	**-1.71**	**9.44**
47.19	**47.35**	**16.55**	**1.48**	**2.07**
16.69	**16.74**	**5.85**	**0.52**	**0.73**
Trial B	Trial B	Trial B	Trial B	Trial B
Muscle Glycogen pre (mmol/g wet)	Muscle Glycogen post (mmol/g wet)	Change (pre to post) (mmol/g wet)	Change (pre to post) (mmol/g wet)	Rate of Muscle Glycogenolysis
120.8118	27.0831	104.14	-346%	13.02
91.1627	28.5756	69.54	-219%	8.69
200.0437	66.0731	148.86	-203%	18.61
116.2123	30.8372	94.86	-277%	11.86
168.4951	15.7614	169.70	-969%	21.21
91.7684	14.8682	85.44	-517%	10.68
195.6414	129.4691	73.52	-51%	9.19
170.9531	51.5397	132.68	-232%	16.59
144.39	**45.53**	**109.84**	**-3.52**	**13.73**
44.66	**38.12**	**36.69**	**2.83**	**4.59**
15.79	**13.48**	**12.97**	**1.00**	**1.62**